Environment Canada Environnement Canada

TOXICOLOGY EXPERT WORKING GROUP REPORT

ENVIRONMENTAL EFFECTS MONITORING TECHNICAL MANAGEMENT COMMITTEE

EEM/1997/5

Canada

Publications relating to Environmental Effects Monitoring (EEM):

Aquatic Environmental Effects Monitoring Requirements (revised EPS 1/RM/18). Environment Canada. 1997. EEM/1997/1

Pulp and Paper Aquatic Environmental Effects Monitoring Requirements. Environment Canada. 1997. Annex 1 to EEM/1997/1

Tracer Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/2 is also published as: Ali, N et al, 1997. Exposed or not exposed: the effectiveness of using tracers to verify exposure of fish to pulp and paper mill effluent. Canadian Technical Report of Fisheries and Aquatic Sciences 2162.

Dioxin Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/3

Usability Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/4

Toxicology Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/5

Fish Survey Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/6

Benthic Community Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/7

Lowell, R.B. Discussion paper on critical effect size guidelines for EEM using benthic invertebrate communities. Report to the Environmental Effects Monitoring Program. EEM/1997/8

Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring. 1998. EEM/1998/1

Workshop on Integrated Approaches for Interpreting Environmental Effects Monitoring Data, Proceedings. EEM/1999/2

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Note

The contents of this report are the views of the members of the Environmental Effects Monitoring (EEM) Expert Working Group. As well, the recommendations in this report are the result of the Expert Working Group process and represent the conclusions of the Expert Working Group.

The recommendations and decision trees put forward in this report will be submitted to the EEM Technical Management Committee (TMC). This information will be used by the TMC to produce the TMC recommendations for updates to the regulatory requirements of the EEM program.

The documents which will contain the revised regulatory requirements will be called:

- Aquatic Environmental Effects Monitoring Requirements, EEM/1997/1
- Pulp and Paper Aquatic Environmental Effects Monitoring Requirements, ANNEX 1 to EEM/1997/1

Acknowledgments

The EEM Technical Management Committee (TMC) extends its thanks to the members of the Expert Working Groups for their contribution to the EEM program. The members of the group have produced their reports in a very tight time frame and they have displayed a willingness to work together to meet common objectives.

The review performed by the Expert Working Groups and their recommendations for enhancements to the program are essential ensuring that the EEM program remains based on sound science.

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1.0 Executive Summary

The following report summarizes EEM Cycle One sublethal toxicity testing results, discusses issues related to sample collection, test methodology and data interpretation, provides recommendations for Cycle Two laboratory toxicity program improvements and recommendations on method validation and research for future EEM cycles. Members of the Expert Working Group (EWG) on Laboratory Toxicology consider Cycle One sublethal toxicity testing to have been successful and recommend that sublethal toxicity remain a core program element for Cycle Two. Cycle One data review provided a unique insight in the benefit of biological effluent treatment as sampling was conducted at several mills before and after the implementation of treatment systems. Even though secondary treatment was designed to eliminate acute lethality and reduce suspended solids & BOD, it also improved effluent quality significantly with respect to the sublethal toxicity. The EWG recommends that all sublethal toxicity tests used in Cycle One be maintained for Cycle Two with the exception of the *Lemna minor* and sheepshead minnow tests. In addition, the EWG recommends for the west coast marine environment that the 7-day topsmelt survival and growth test be added as a test option. The EWG recommends an increase in the frequency of sublethal toxicity testing and has provided two options with respect to frequency of testing for Cycle Two. Option one (12 samplings/cycle) recommends that tests be performed four times a year over the three-year cycle and option two (6 samplings/cycle) recommends that tests be performed two times a year over the three-year cycle. Rationale is provided for both options. The EWG has also agreed that the specific objectives for sublethal toxicity testing be clearly stated in Annex 1.

The EWG has recommended method refinements and clarifications to specific toxicity tests used in Cycle One and has suggested specific changes be made to EEM toxicity data interpretation guidance. In addition, the EWG recommended that research be conducted to better define the laboratory-to-field relationship to attain greater confidence in the predictive capability of laboratory toxicity tests.

2.0 Introduction

2.1 Working Group Purpose, Specific Objectives, and Membership

Beginning in 1992, all Canadian mills regulated under the amended Pulp and Paper Liquid Effluent Regulations were required to conduct an Environmental Effects Monitoring (EEM) program at regular intervals in accordance with Sections 28 to 35 of the regulation, the generic EEM requirements document (ie: EPS 1/RM/18) and specific requirements outlined in Annex 1 of the EEM requirements document. The overall objective of the EEM program is to assess the adequacy of national regulations for protecting fish, fish habitat and the use of the fisheries resources. The results of the first cycle of EEM monitoring program were provided to the Federal Government in the form of an interpretative report on April 1, 1996.

In June 1996, the Technical Management Committee (TMC) established Expert Working Groups to: 1) assess the data produced in the course of Cycle One of the EEM program; 2) to examine sampling and methodology issues; and 3) to provide specific recommendations on the requirements to be applied in Cycle Two. In addition, the EWGs were asked to provide guidance to the TMC on

long-term issues such as future research for the purpose of improving the quality of the data, monitoring tools, and data interpretation. The EWG on Laboratory Toxicology, building on a generic Terms of Reference supplied by the TMC, produced a more specific Terms of Reference for their group which focused on issues relating to the sublethal toxicity data generated during Cycle One and on possible improvements to this component of the EEM program.

The following issues were to be addressed by the EWG (also see Appendix A for complete Terms of Reference):

- review sublethal toxicity data from Cycle One;
- identify difficulties related to sample collection and test methodology;
- discuss the adequacy of the frequency of sublethal toxicity testing;
- discuss the ability of sublethal toxicity data to detect changes in effluent quality;
- discuss current guidance on interpreting EEM sublethal toxicity data;
- discuss possibility of introducing alternative tests and criteria for new test acceptance;
- discuss whether general conclusions can be made with regards to the relationship between laboratory sublethal toxicity test data and observed field effects.

Members of the EWG on Laboratory Toxicology were representatives drawn from industry and government and were selected on the basis of either their expertise in laboratory toxicology and/or their knowledge of the EEM program. The EWG was co-chaired by a government and industry representative to achieve a balanced view in the resulting recommendations. The names and affiliations of EWG members who actively participated in the discussions and preparation of this report are as follows:

Pierre Martel (co-chair)	PAPRICAN, Environmental Quality Research, Pointe Claire, Quebec
Richard Scroggins (co-chair)	DOE/ETC, Method Development & Application Section, Ottawa, Ontario
Kenneth Doe	DOE, Toxicology Laboratory, Dartmouth, Nova Scotia
Barry Firth	Weyerhaeuser, Environmental Science & Technology, Tacoma, Washington
Katsuji Haya	DFO, Marine Environmental Sciences Div., St. Andrews, New Brunswick
Jennifer Miller	DOE/ETC, Method Development & Application Section, Ottawa, Ontario
Valerie Naish	Domtar, Innovation Centre - Environment, Ste-Anne de Bellevue, Quebec
Joanne Parrott	DOE/NWRI, Aquatic Ecosystem Conservation Branch, Burlington, Ontario
Jerry Payne	DFO, St. John's, Newfoundland
Danielle Rodrigue	DOE/ETC, Method Development & Application Section, Ottawa, Ontario
Jim Sherry	DOE/NWRI, Aquatic Ecosystem Conservation Branch, Burlington, Ontario
Donald St-Laurent	DOE, Environmental Protection Branch, Montreal, Quebec
Michel Taché	DOE/HQ, EEM National Office, Hull, Quebec
Graham van Aggelen	DOE, Aquatic Toxicology Laboratory, Vancouver, British Columbia
Gary Westlake	MOE&E, Aquatic Toxicology Laboratory, Rexdale, Ontario

2.2 History of Working Group Activities and Process

The EWG on Laboratory Toxicology started its activities in July 1996 and met in person or via teleconference on seven occasions (ie: July 8, September 30 and November 25/26 of 1996, January 16/17, February 12/13, March 11/12/13 and April ½ of 1997) in Montreal, Ottawa or Toronto. Discussions were focused on Cycle One data and issues arising from Cycle One. The results of these discussions have been recorded in six sets of minutes which were distributed to all stakeholders of the EEM program following approval. The following document is based on the discussions and decisions recorded over the course of the previous meetings. The EWG strived to come to a consensus on as many issues as possible to provide clear recommendations for Cycle Two.

3.0 Discussions of Issues and Proposed Solutions

3.1 Review of Cycle One Sublethal Toxicity Data and Guidance

3.1.1 Summary of Requirements and Sublethal Toxicity Data

The sublethal toxicity test methodologies for Cycle One are outlined in Table 1 (originally from Section 10 of Annex 1). At all mills, a battery of three sublethal toxicity tests using a fish, an invertebrate and a plant species were conducted on samples of final mill effluent collected during four consecutive quarters within the principal year of field work. Sublethal testing was completed on the final effluents of 123 mills across Canada. To allow EWG discussion on the effectiveness of Cycle One sublethal toxicity, a national summary of the data was prepared by extracting the IC25 results from each mill's Cycle One interpretative report. EEM program staff located at Environment Canada regional offices extracted the test data from each interpretative report, verified the accuracy of these data, and prepared summary spreadsheets. These data are presented in Appendix B as Table B.1 "Results of Sublethal Marine Tests for Cycle One EEM (Inland silverside, Echinoderm, *Champia*)"; Table B.2 "Results of Sublethal Freshwater Tests for Cycle One EEM (*Ceriodaphnia, Selenastrum, Lemna*)"; and Table B.3 "Results of Sublethal Freshwater Tests for Cycle One EEM (Fathead minnow, *Oncorhynchus*)".

Table 1: Methodologies for EEM Cycle One Effluent Toxicity Testing

Test Description	Receiving Environment	Test Species	Acceptable Method(s)
Fish early life stage development test	Marine	Sheepshead minnow or Inland silverside	U.S. EPA 1991
	Freshwater	Fathead minnow or Salmonid spp.[1]	Environment Canada 1992c or Environment Canada 1992g
Invertebrate reproduction test	Marine	Echinoids (sea urchins or sand dollars)	Environment Canada 1992d
	Freshwater	*Ceriodaphnia dubia*	Environment Canada 1992b
Plant toxicity test	Marine	*Champia parvula*	U.S. EPA 1991
	Freshwater	*Selenastrum capricornutum* or *Lemna minor*	Environment Canada 1992c or APHA 1992

[1]For Canadian receiving environment locations where fathead minnows are not an indigenous species, a salmonid species must be used according to Environment Canada (1992g)

3.1.2 Sublethal Testing Successes and Difficulties

Successes

The mills and their consultants completed all Cycle One sublethal toxicity testing on schedule and the quality of the data generated was good. All EWG members agreed that: 1) the data set from Cycle One was limited to four sublethal test results generated per mill during the cycle which may restrict statistical analysis and/or interpretation of the data; 2) at many mills, there was significant variation among the four toxicity test results (conducted quarterly) due to variability of effluent quality, test method variability and/or changes in effluent quality due to significant improvements in mill process or effluent treatment during the cycle; 3) there were specific sample collection and test methodology issues to be resolved; and 4) it was recognized that Cycle One was not designed to identify correlations between laboratory and field data.

The Cycle One national data set for each sublethal toxicity test is summarized in Figures 1 and 2 for freshwater tests and in Figure 3 for marine/estuarine tests. The data has been divided into primary- or secondary-treated effluent categories for each of the eight toxicity tests. The EWG decided that the performance of each sublethal toxicity test should be evaluated using two main criteria: 1) ability of the test to measure effluent quality over a broad range of test concentrations; and 2) ability to distinguish a trend towards improved effluent quality as a result of secondary treatment installation. Even though secondary treatment was designed to eliminate acute lethality and reduce suspended solids and BOD, it also improved effluent quality significantly with respect to the sublethal toxicity. Some members cautioned that categorizing all effluent toxicity data by primary and secondary treatment for the purpose of determining the impact of effluent treatment

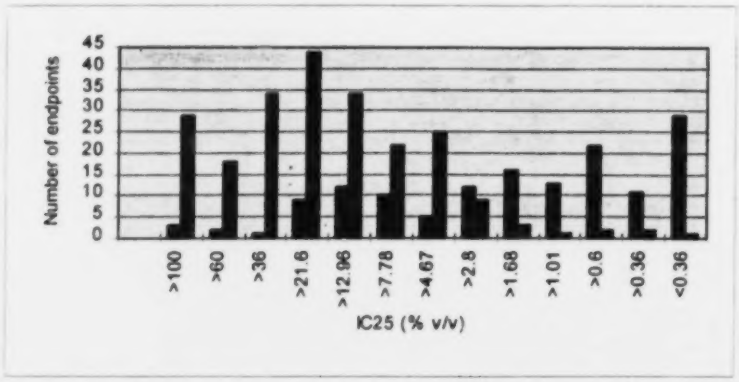
improvements needs to be qualified since the specific type of treatment, treatment system performance and mill process type are not considered in the summary figures. Ideally, the effect of secondary treatment on sublethal effluent toxicity should be examined on a mill by mill basis. The EWG consensus view on the effectiveness of the Cycle One sublethal toxicity tests is summarized in Table 2. EWG members concluded that, with the exception of *Lemna minor*, most sublethal toxicity tests were sufficiently sensitive.

Table 2: Effectiveness of Sublethal Toxicity Tests Used in Cycle One

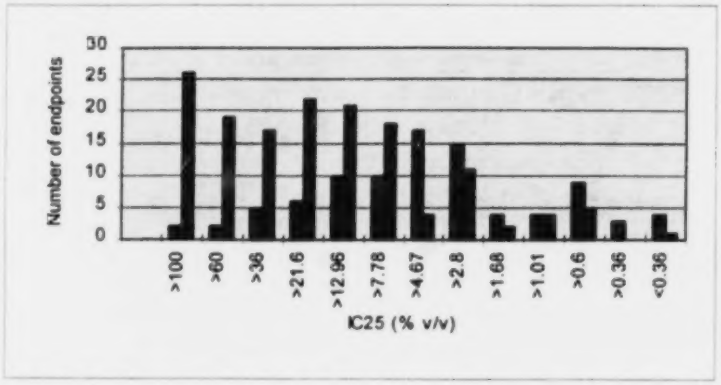
Test Method	Sensitivity[1]	Trend[2]
Ceriodaphnia dubia	+	+
Selenastrum capricornutum	+	+
Lemna minor	-	-
Fathead minnow	+	+
Trout embryo	+	Insufficient data
Echinoderm	+	-
Champia parvula	+	-
Inland silverside	+	+
Sheepshead minnow	•	•

[1] = Ability of tests to measure effluent quality over a broad range or test concentrations
[2] = Ability to distinguish a trend towards improvement with secondary treatment installation
• = Not rated since the sheepshead minnow test option was not chosen by any marine or estuarine discharging mill in Cycle One

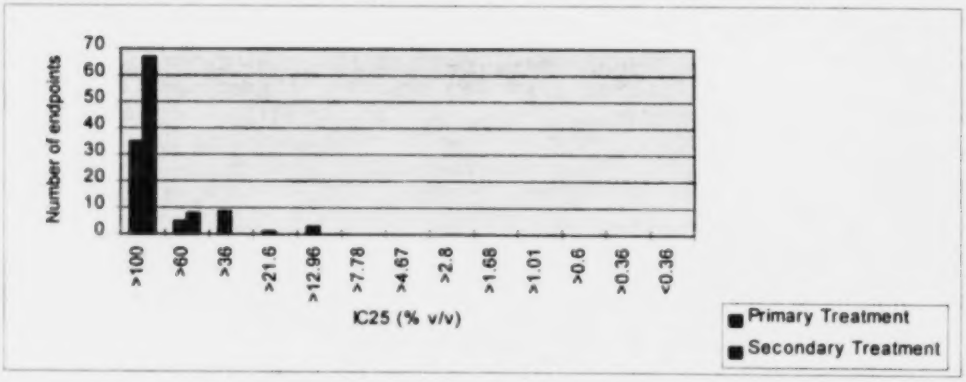
Figure 1: Cycle One Data for Three Freshwater EEM Sublethal Toxicity Tests



Ceriodaphnia Reproduction Data

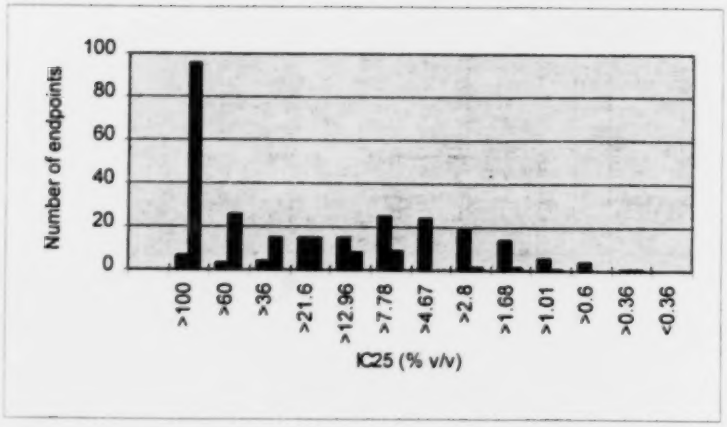


Selenastrum Growth Data

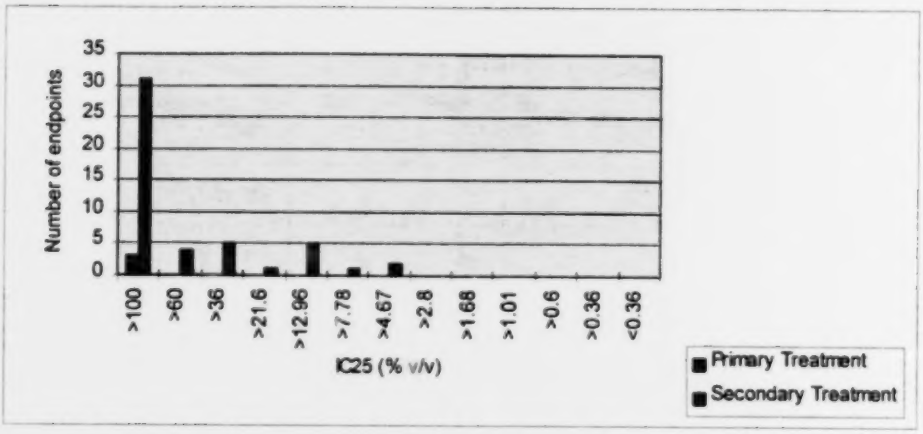


Lemna Growth Data

Figure 2: Cycle One Data for Two Freshwater EEM Sublethal Toxicity Tests

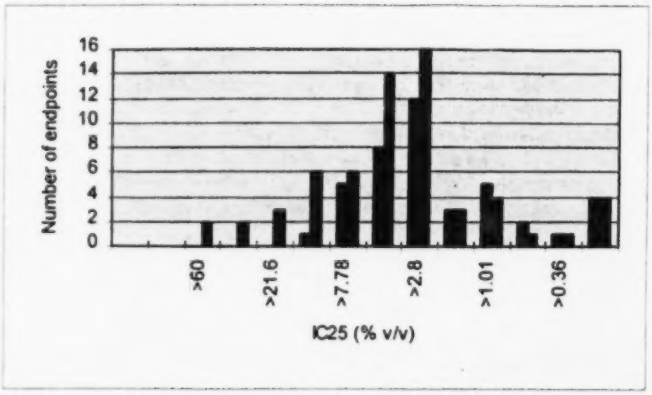


Fathead Minnow Growth Data

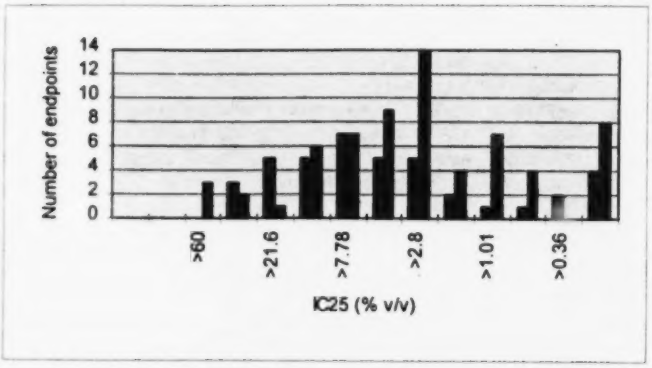


Early Life Stage Salmonid Survival Data

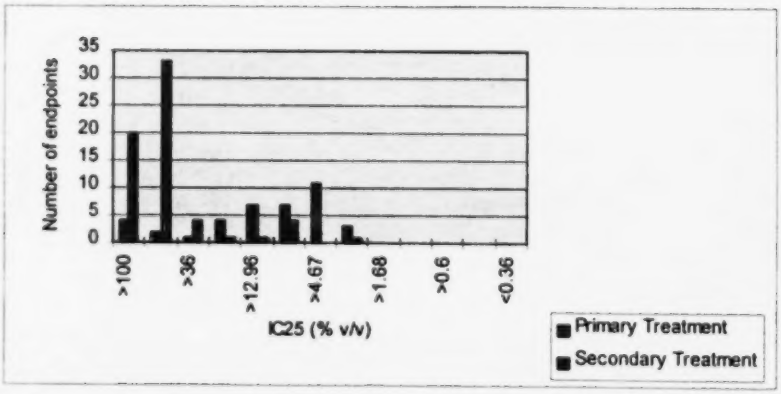
Figure 3: Cycle One Data for Marine EEM Sublethal Toxicity Tests



Champia Reproduction Data



Echinoderm Fertilization Data



Inland Silverside Growth Data

Difficulties

Based on Table 2, the EWG felt that two tests (ie: *Lemna minor* and sheepshead minnow) had not performed adequately. These tests are not recommended for use in Cycle Two. The principal reasons for discontinuing the *Lemna minor* test were lack of a properly standardized procedure, poor ability to distinguish effluent quality changes and lack of sensitivity. With regards to the trend criterion, some tests clearly showed the trend towards improved effluent quality with secondary treatment (ie: *Ceriodaphnia*, *Selenastrum*, fathead minnow, inland silverside) while improvement was not as obvious with other tests (ie: echinoderm and *Champia*). It was also felt that there was not enough data to assess the ability of the trout embryo test to detect a trend in effluent quality.

During its seven meetings, the EWG discussed many issues and recommendations that were identified in regional government/industry workshop reports, in industry position papers, by Environment Canada regional staff and from environmental consultants who conducted the EEM studies for the pulp and paper industry. Issues and recommendations specific to Cycle One sublethal toxicity testing have been extracted from various workshop and meeting reports and reproduced in Appendix C. A summary of issues is provided in Table 3 with issues that were most frequently mentioned listed first. The most common issues identified were insensitivity of the *Lemna minor* growth inhibition test and the frequency of sublethal testing. All advice received on the *Lemna minor* test supports the EWGs recommendation that this test be removed as an option in Cycle Two.

Advice on the frequency of testing issue was mixed. EWG discussion on the frequency of testing issue was substantial and, following attempts to find a consensus position, ended with two options for the EEM Technical Management Committee to consider. A summary of the discussion which lead to the two differing viewpoints is provided in Subsection 3.1.4.

The next most common issue identified was the need to explore the relationship between laboratory toxicity and observed field effects data. This issue was the subject of considerable debate during a number of meetings and also lead to different viewpoints on the ability of the current battery of sublethal toxicity to predict effects in the field. This discussion has been summarized in Section 3.4. It is notable that four out of five industry/government EEM workshops recommended that sublethal toxicity testing remain a core requirement for Cycle Two. All EWG members supported this position as well.

The EWG agrees that too many toxicity test endpoints were required in Cycle One and is recommending that only point estimation endpoints be required for Cycle Two tests (ie: IC25, EC25 and LC50 and associated 95% confidence intervals as appropriate). On the issue of *Champia* test method variability, the EWG recommends that laboratories initiate some of the method improvements suggested during their meetings (ie: use of two male branches to ensure adequate gamete availability and use of a device to provide gentle agitation of test solutions).

Issues Related to Test Methodology Quality Assurance and Quality Control (QA/QC)

Several difficulties seen in Cycle One data could have been avoided by better adherence to good QA/QC practices. Suggestions were discussed by the EWG for consideration in Annex 1. These changes focus on ensuring that valid toxicity data of high quality will be generated by private testing laboratories during Cycle Two.

For example:

- reference toxicant tests must be similar to the effluent test;
- a specific time interval within which an associated reference toxicant test must be initiated;
- a statement indicating that tests that fail to meet validity criteria must be repeated;
- mandatory compliance to effluent sample age limit; and
- laboratories conducting Cycle Two tests have CAEAL, MEF, or equivalent level of accreditation.

The EWG also agreed that quality assurance checklists should be prepared to help mill and regional office EEM staff ensure that valid data is generated by the testing laboratory.

At least one industry/government EEM workshop recommended the use of upstream or reference site receiving water as dilution water for sublethal toxicity tests to increase the relevance of the tests. While Cycle One was being designed in 1991, there was a discussion on the pros and cons of using site water but the idea was rejected because pulp and paper effluent is less influenced by water quality. Going into Cycle Two, all mills will have secondary effluent treatment and the EWG felt that using receiving water for test controls and dilutions would be even less justifiable from an interpretation and increased test cost point of view.

The EWG addressed a number of generic and specific test method issues identified primarily by the private consulting laboratories who carried out Cycle One testing and recommendations on these issues are outlined in Section 3.2.

The EWG discussed the history, purpose and activities of the national laboratory accreditation program managed by the Canadian Association for Environmental Analytical Laboratories (CAEAL). The CAEAL program is voluntary, follows International Standards Organization (ISO) quality assurance standards, and is funded by member laboratories (ie: accreditation is granted after the laboratory has met performance testing and laboratory inspection requirements). There are currently 64 voluntary auditors that have completed the ISO auditor training program. The focus of the audits (typically 2-3 days in duration) is to review the laboratory's Quality System specifically focusing on the Quality Manual, individual test Standard Operating Procedures (SOPs) and interviews with the quality manager and key lab technicians.

The B.C. government has an agreement with CAEAL in that all participating laboratories must be CAEAL certified (ie: participate in round robin performance testing only) to generate permittee data within the Province of British Columbia. The "Ministère de l'environnement et de la faune du

Québec" (MEF) has a similar accreditation program to CAEAL's program which is also based on ISO 9002 and Guide 25 standards. Although the use of CAEAL accredited laboratories was premature for Cycle One, there was general agreement by EWG members that laboratories with CAEAL, MEF or an equivalent level of accreditation should be hired by mills for Cycle Two.

Table 3: EEM Laboratory Toxicology Issues Identified by Industry, Government and Consultants

	Industry/Government EEM Workshops					AFPA[1] Paper	Environment Canada Regions				Cons[2]
Issues	**SID[3]**	**DWN[4]**	**FRED[5]**	**TO[6]**	**MTL[7]**		**ATL[8]**	**QUE[9]**	**ONT[10]**	**P&Y[11]**	**ST. AND[12]**
Test insensitivity *Lemna minor*	X	X	X		X		X	X	X		
Test frequency	X		X	X	X	X	X			X	
Relationship between lab and field			X	X	X		X	X	X		
Keep lab. tox. tests as core element		X	X	X	X		X		X		
Champia test improvement	X		X				X	X			X
Too many test endpoints		X						X	X		X
Sample age									X	X	X
Requirement for use of PMTC	X		X		X						
Use of receiving water in tests				X	X			X			
Conformity to validity criteria									X	X	
Frequency of ref. toxicant testing							X	X			
Reference toxicant endpoint										X	X
Effluent characterization	X									X	
Decision criteria	X									X	
Test insensitivity fathead minnow						X		X			
Effluent toxicity loading						X					X
Failure to re-test when invalid								X		X	
Test insensitivity inland silverside	X							X			
Need for QA / QC checklist				X	X						
²Must² requirements not respected										X	
Reporting deficiencies							X				
Rationale for conducting tests				X							
Trout E-Test: egg availability	X										

[1] Alberta Forest Product Association
[2] Consultants
[3] Sidney
[4] Downsview
[5] Fredericton
[6] Toronto
[7] Montreal
[8] Atlantic
[9] Quebec
[10] Ontario
[11] Pacific & Yukon
[12] St. Andrews

Electronic Reporting to Simplify Toxicity Data Review

The EWG recognized the complexity of the review process at the end of Cycle One. To help alleviate the problem of all data being reported at the end of the cycle, the EWG recommends that the mills be required to submit the results within 60 days of completing the test and that the government review be completed within 60 days following reception of the results. This would allow for early identification of problem data, and retesting where necessary. Furthermore, EWG members agreed that if the sublethal test results were submitted electronically in a common format, the process of data review would be simpler, could be completed in less time, and would make historical data more accessible. As the reporting requirements of the Environment Canada toxicity test methods are being updated, there is an opportunity to also develop a consistent and common electronic toxicity data reporting system. In order to ensure reasonable time frames for reporting and reviewing of sublethal data, an electronic mechanism is essential.

A computer database consultant working with a toxicologist could implement such a database and data entry system for sublethal toxicity test results in time to receive the first data of Cycle Two. The EWG recommends that this work be done as a demonstration pilot project and estimates a one time cost as for implementation is $20K and operating costs of $5K per year. The following are among the considerations in developing an electronic reporting system for sublethal data:

- the added cost to each toxicity test result needs to be estimated and compared to the cost savings of reporting and review;
- the system must be easy enough to use, clearly and simply documented to be practical for the intended users;
- the system should be PC-based and professionally designed;
- the system should be as compatible as possible with existing databases used by toxicity laboratories, industry and government agencies; and
- the data should be entered once only and the program should be made freely available.

The project would require the purchase of one microcomputer with modem and data quality phone line. The microcomputer would not have to be special but should have mirrored drives with CD and tape back up for data security. The programmer would design and install the database with the advice of the toxicologist. Data entry screens and reports would also have to be designed. When operational, the microcomputer would receive calls from consultants or companies which would be followed by a call back for security purposes and to assign long distance charges to the EEM program. Access could be restricted even to the field level of the database if desired.

3.1.3 Objectives of Sublethal Toxicity Testing within EEM

The EWG felt that the current objectives for the use of sublethal toxicity tests were unclear and for Cycle Two, the objectives should be stated clearly in Annex 1 and in EEM program guidance documents.

As a first step, an attempt was made to identify the current objectives for the use of sublethal toxicity tests from various EEM Cycle One documents. It was apparent that Annex 1 did not provide specific objectives for sublethal toxicity testing. Specific rationale for sublethal toxicity testing were found in sentences contained in Sections 5.1.2 and 5.1.3 of the "Guidance on the Interpretation of Sublethal Toxicity Test Data Generated for the EEM Program under the Pulp and Paper Effluent Regulation" which was distributed to industry by Environment Canada's regional offices in October 1995. These objectives were:

5.1.2, Paragraph 3: "The obvious first use of the tests is to provide a rating of an effluent's toxicity, both over time and with respect to other effluents."

5.1.3, Paragraph 1: "The sublethal test results can also be used to assess the likelihood of toxic effects in the receiving water. At its simplest, such an assessment might compare the endpoints from the tests (IC25 or TEC) with concentrations expected in the plume where the effluent mixes into the receiving water".

There was unanimous agreement within the EWG that the sublethal tests can be used to assess effluent toxicity over time (eg: before and after process or treatment system changes). Some EWG members felt that sublethal toxicity tests could be used to assess relative contributions from various effluent discharges in the same vicinity while others felt that this was only a possibility. However, EWG members had differing views on whether sublethal toxicity tests can be used to predict potential field effects.

Within the EWG, there was more than one point of view concerning the use of tests for prediction purposes. Some members felt that the purpose of the EEM program is environmental monitoring in the field and not end of pipe. The sublethal toxicity tests can provide additional information on effluent quality and are only a coarse measure of response. Better linkage to the field was a desirable goal but not appropriate for an objective for Cycle Two but should be defined as a research need. Other members felt that we should be working towards designing a program with predictive capability for the future, and that prediction is the underlying basis for the use of toxicity tests. However, these same members indicated that some of the current sublethal toxicity tests appeared to have predictive capability.

The EWG discussed the views of objectives for the use of sublethal toxicity tests within EEM tabled by industry members. These were:

- to rate effluent toxicity over time;
- to possibly differentiate other sources; and
- to contribute to the field program as part of the weight of evidence approach rather than prediction.

Another set of objectives for the use of sublethal toxicity tests in Cycle One was outlined in Environment Canada's presentation at the EEM Symposium held in conjunction with the Aquatic Toxicity Workshop in St. Andrews, New Brunswick, October 1995. These objectives were

outlined as part of the presentation introducing Cycle One guidance for interpretation of sublethal toxicity data. The three objectives were that sublethal toxicity tests:

- can estimate the zone of potential effects in a receiving water body through measurement of sublethal toxicity on samples from the mill's effluent discharge;
- can help define whether or not observed receiving environment effects are linked to a particular mill effluent discharge as opposed to toxicity input(s) to the same receiving water system from other point or non-point sources; and
- can quantify improvements in effluent quality over time.

During the January EWG meeting, many of the EWG members agreed with the above three main objectives for the use of toxicity tests within EEM.

During the March meeting, the EWG discussed the rationale for conducting sublethal toxicity tests suggested by industry/government regional coordinators at their meeting in February 4, 1997. Using the rationale from the minutes of this meeting, the EWG came to a consensus on the three objective statements below:

- to compare effluent quality between mill types and to measure changes in effluent quality as a result of effluent treatment and process changes;
- to determine the relative contributions of multiple discharges;
- to assess the potential impact of an effluent on the receiving environment.

To ensure the objective statements were clearly understood and not misinterpreted, it was agreed that further explanation be prepared to accompany the three criteria and that the criteria and supporting paragraph be incorporated in the revised Annex 1 and interpretative guidance document. The consensus paragraphs supporting the objectives are as follows:

"The EEM program is designed to assess the adequacy of the national effluent regulation in protecting fish, fish habitat and the fisheries resource. This is done by fish surveys, benthic community surveys, laboratory toxicology, and measurement of dioxins and tainting in fish. Sublethal toxicity measurements are essential to determine biological effects of effluents, now that regulatory requirements for BOD, suspended solids, and acute lethality have been achieved. The response of aquatic organisms to effluents obtained under controlled conditions can be used to quantify and compare the toxicological characteristics of mill effluents over time. Similarly, these tests could be used to compare potential contributions of discharges from multiple sources.

Within the current limits of our understanding of the relationship between biological responses measured in the laboratory and those measured in the field, laboratory toxicity measurements may provide an indication of effects in the environment. For example, an improvement in effluent quality shown by toxicity data suggests that we should expect an improvement in the aquatic environment. If the results of future research show that toxicity tests have the ability to predict specific effects on wild organisms, then those tests will be helpful in assessing the potential impact of pulp and paper mill effluents".

Further to the last EWG meeting, two members suggested additional wording modifications to the above consensus paragraphs and these suggestions are presented in Appendix D.

3.1.4 Adequacy of Testing Frequency

In Cycle One, the frequency of sublethal toxicity testing on mill effluent was four times during the principal year of field work. When the EWG first initiated discussion on the adequacy of the current frequency of testing, initial positions taken by individual members were directly related to their view of the principal rationale for conducting sublethal toxicity testing within the EEM program. Basically, most members felt that the number of testing periods within a cycle was dependant on which rationale was of primary importance. EWG views on the objectives for the use of sublethal toxicity tests in the EEM program are discussed in Subsection 3.1.3.

Some members felt that there were too few data points for each toxicity test in Cycle One to obtain a good estimate of the potential zone of effect in the receiving environment and to get a good appreciation of sublethal toxicity effluent variability. Other members felt that the principal use for sublethal toxicity tests in the EEM program was to quantify improvements in effluent quality over time. At the January EWG meeting, each meeting participant was asked to comment on the number of effluent testing periods needed to provide sufficient sublethal toxicity data for Cycle Two and timing of this testing. A summary of meeting participant views is presented in Table 4.

Table 4: Summary View of January EWG Meeting Participants on Testing Frequency

	Number of Effluent Samples per Typical Cycle*	Total Number of Tests per Typical Cycle*	Timing During Cycle*
6 Participants	12	36	quarterly every year
4 Participants	4	12	quarterly in year of field work
2 Participants	more than 4 (not specific)	12 +	quarterly every year
1 Participant	6	18	twice per year

* Assuming a typical cycle is 3 years

At the February EWG meeting, there was an attempt to reach a compromise on the frequency of testing issue. There were five propositions tabled as part of the attempt to reach a common recommendation.

Proposition #1: 4 samples per field work year, for a total of 4 samples per typical three year cycle (status-quo);

Proposition #2: 6 samples, 2 per year over a typical three year cycle;

Proposition #3: 6 samples (consecutive quarters) per typical three year cycle, centred on the main year of field work;

Proposition #4: 8 samples per typical three year cycle (2 the first year, 4 the second year and 2 the third year);

Proposition #5: 12 samples per typical three year cycle, conducted each quarter.

Although all EWG members supported an increase in the frequency of sublethal toxicity testing, the members were almost equally split between propositions for 6 and 12 testing periods per typical EEM cycle. Attempts to reach consensus was partially successful in that a number of members moved from their initial status-quo position of 4 testing periods to 6 testing periods per cycle. As well, some members who favoured 12 testing periods were willing to compromise on 8 testing periods per cycle. As further compromise did not seem possible, the EWG agreed to present two options to the TMC: 1) 6 testing periods with testing occurring twice per year over a typical cycle; and 2) 12 testing periods with testing occurring four times per year over a typical cycle. The EWG also agreed to prepare supporting rationale for both options to assist the TMC in reaching a decision. It was suggested that the rationale for the differing positions should not emphasize an economic argument.

The rationale for 6 testing periods per cycle is:

- better continuity over the duration of cycle;
- the increase in cost is acceptable in view of the information gained;
- more efficient use of laboratory resources in consulting firms;
- facilitates the process of submission and review of data for errors and timing is such that errors can be corrected before subsequent sampling;
- data gathered in Cycle Two and One will provide a strong basis to determine appropriate testing frequency in Cycle Three;
- harmonization with existing provincial requirements in Ontario.

The rationale for 12 testing periods per cycle is:

- 12 data points per test over a three year cycle is a reasonable number for estimating extent of effluent sublethal toxicity variation;
- 4 testing periods per year would provide the minimum data set needed for estimation of seasonal variation in effluent sublethal toxicity;
- for estimation of potential zones of effect, 12 data points per cycle would be sufficient to allow a reasonable estimate of the lowest IC25 value likely to occur and preclude the need for a PMTC statistical estimate;

- 12 data points per sublethal toxicity test is a reasonable balance between cost and sufficient data to estimate mill-specific and national trends for secondary-treated effluent quality for Cycle Two;
- generation of sufficient sublethal toxicity data to establish effluent quality trends with a high degree of confidence is a more appropriate approach (ie: precautionary approach). After this trend has been established, consideration should be given to a reduction in the frequency of sublethal toxicity testing.

The EWG felt that realistic costs for frequency of testing options should be prepared to assist the TMC in making their choice. Table 5 outlines the average cost for sublethal toxicity tests (*Selenastrum*, *Ceriodaphnia*, fathead minnow, salmonid E-test, *Champia*, echinoderm, inland silverside) based on quotations from six Canadian CAEAL/MEF accredited laboratories. Based on these estimated costs, cost scenarios for 4 , 6 , 8 and 12 testing periods per cycle per mill and the incremental cost increase above the 4 testing period status-quo option are outlined in Tables 6 and 7, respectively.

EWG members agreed that a decrease in testing frequency should be considered after Cycle Two for mills that perform well (despite uncertainty as to how to establish the criteria or trigger for this decrease).

Table 5: Quotation from CAEAL/MEF Accredited Laboratories for Sublethal Toxicity Tests

Method	Number of Quotes per Test	Cost/Sample (<5 samples/year)	Cost/Sample (>5 samples/year)
Freshwater *Selenastrum*	5	$513	$469
Ceriodaphnia	6	$977	$903
Fathead minnow	6	$1077	$1003
Salmonid E-test	4	$1735	$1712
Marine *Champia*	3	$1760	$1715
Echinoderm	3	$1140	$1117
Inland silverside	3	$1510	$1483

Table 6: Cost Scenarios per Mill

Scenario	Freshwater	Marine
STATUS QUO: 4 samples within the year of the field work (total 4)	$ 10,268. (with FHM) $ 12,900. (with E-test)	$ 17,640.
Twice a year per cycle (total 6)*	$ 15,402. (with FHM) $ 19,350. (with E-test)	$ 26,460.
Total of 8 per cycle*	$ 20,536. (with FHM) $ 25,800. (with E-test)	$ 35,280.
Four times a year per cycle (total 12)*	$ 30,804. (with FHM) $ 38,700. (with E-test)	$ 52,920.

* Assuming a typical cycle is 3 years FHM = Fathead minnow

Table 7: Potential Costs for Sublethal Toxicity Testing in Cycle Two for the Pulp & Paper Industry by Scenarios

Scenarios*	Status Quo (4 samples/cycle)		Incremental Increase		Total Cost
6 samples/cycle	$1,483,060.	+	$741,530.	=	$2,224,590.
8 samples/cycle	$1,483,060.	+	$1,483,060.	=	$2,966,120.
12 samples/cycle	$1,483,060.	+	$2,966,120.	=	$4,449,180.

* Assuming 121 mills which are participating in EEM: 28 marine mills and 93 freshwater mills (80 with fathead minnow and 13 with the E-test)

3.1.5 Are Decision Criteria for Sublethal Toxicity Data Required for Cycle Two?

The EWG considered the need for criteria to guide the review and interpretation of sublethal toxicity test results that will be or to be generated during Cycle Two. The view of some EWG members was that sublethal data are not being collected to be just filed away for posterity, and some interpretation of the data will be required. Although the objectives for the use of laboratory toxicity tests in the EEM program has been clarified in Section 3.1.3, decision criteria should guide the interpretation of the data within the framework of those objectives. Following lengthy discussions, the consensus among EWG members was that the decision criteria should be intended to aid the interpretation of Cycle Two data but not be rigid or overly specific. As principle, EWG members agreed that laboratory toxicity data should be used to support field data in a weight-of-evidence approach.

The EWG discussed the fact that the U.S. EPA has developed guidance and has implemented decision criteria for the use of sublethal toxicity tests. The EPA and various U.S. States have set compliance limits for sublethal toxicity which consider State policies related to setting the minimum low flow dilution, the designated use of the receiving water body, and the allowed regulatory mixing zone. The EPA's recommended decision criteria, however, are not uniformly applied across the United States. Some EWG members expressed concern about the use of the U.S. EPA's guidance as a template for decision criteria. The consensus was that interpretation of the sublethal toxicity data should be done on a site specific basis.

The term "Decision Criteria" can be interpreted in a number of ways, one interpretation is encapsulated in the following question: "How do we decide from the results of the tests for sublethal toxicity if an impact on the environment is a possibility ?" The sublethal toxicity tests can also play a role in sorting out confounding factors such as the relative contributions of discharges from multiple sources. Some EWG members were of the opinion that an impact on the relevant component of the biota could be predicted for a zone of influence within which the in-stream effluent concentration exceeds the IC25 value from the laboratory toxicity test.

Inherent in this position is an understanding that laboratory sublethal toxicity tests have the potential to predict relevant sublethal effects in similar organisms in the field. Environment Canada's 1995 guidance document suggests a predictive role for sublethal toxicity tests in the EEM program (ie: Sections 5.1.3 and 5.1.4). In an ideal situation, a laboratory toxicity test should predict an observable effect, that is mechanistically linked to the test's end-point, in the receiving water body. The 1995 guidance document was intended to provide guidance on data interpretation to the industry and their consultants and was not a legal requirement. Some EWG members felt that, given the current state of the science, research is needed to improve the predictive capability of sublethal toxicity tests.

Concern was expressed that even if the results of laboratory toxicity tests at some sites are useful for predicting effects whether real or potential, or triggering a set of decision criteria in situations where the mill's effluent makes up a significant portion of the receiving water body, they may not be applicable to other situations. Often it is difficult to establish the minimum low flow in the receiving water body, and as a result, the calculation of dilution rates and impact zones becomes a problem.

A different opinion expressed by other members was that there was no need for the EWG to establish sublethal toxicity decision criteria since the EEM program is primarily a field monitoring program and that there are no situations in which sublethal toxicity tests could predict potential field affects. Their view was that current laboratory toxicity tests can be used to detect effluent quality changes, but are not predictive of field effects.

It was also the opinion of some EWG members that Environment Canada's current guidance for the interpretation of sublethal toxicity data (ie: particularly Section 5.1.4) was adequate and there is no need for more specific guidance on decision criteria for Cycle Two.

3.1.6 Review of Data Interpretation Approaches

The EWG reviewed the data interpretation guidance prepared by Environment Canada in October 1995 and recommends that the guidance be modified to describe three possible options for the estimation of potential zones of effect:

- using the lowest IC25 value available from sublethal toxicity tests conducted on the mill's final effluent;
- using the geometric mean of the IC25s generated from sublethal toxicity tests conducted on the mill's final effluent; or
- using the Predicted Minimum Toxic Concentration (PMTC) statistical procedure.

The EWG acknowledged that use of the geometric mean of the IC25 results and the PMTC statistical procedure for estimating potential zones of effect at individual mill discharge sites was not a mandatory requirement for Cycle One. This approach to applying EEM sublethal toxicity test results was recommended in Environment Canada's October 1995 guidance document on interpreting sublethal toxicity data under the pulp and paper EEM program. The PMTC is a statistical procedure applied to toxicity data to predict the lowest IC25 that would likely be encountered or, in other words, the concentration of effluent below which 5% of the samples would be expected to have a deleterious sublethal effect as estimated at the 95% level of confidence. The guidance recommends the potential zones of effect be defined as the area of the plume in which concentrations exceed the geometric mean of the IC25s or the PMTC value.

Mean IC25s and PMTCs were calculated using Cycle One sublethal toxicity data from each mill with the exception of mills which generated less than three valid tests or if there was a major change in effluent quality due to secondary treatment coming on-line after two testing periods. As well, where secondary treatment came on-line prior to the 4th quarter testing period, the 4th quarter data point was excluded from the PMTC calculation. As an example, mean IC25 values and calculated PMTC for *Ceriodaphnia* reproduction data from all freshwater mills are compared in Figures 4 and 5 for mills with primary and secondary effluent treatment respectively. The PMTC values for mills with only primary treatment (Figure 4) were highly affected by variability in toxicity between effluent samples collected at individual mills as well as by low numbers of toxicity test results available for the calculation (ie: 3 or 4 data points only). In some cases, the difference between the mean IC25 and the PMTC estimates for mills with primary treatment was six orders of magnitude. However, for mills with secondary treatment on-line (Figure 5), the difference between the mean IC25 and the PMTC estimates was typically only three orders of magnitude. So, reduced variability as a result of better effluent treatment was the main reason behind the reduction of the difference between mean IC25 and the PMTC estimates for *Ceriodaphnia*.

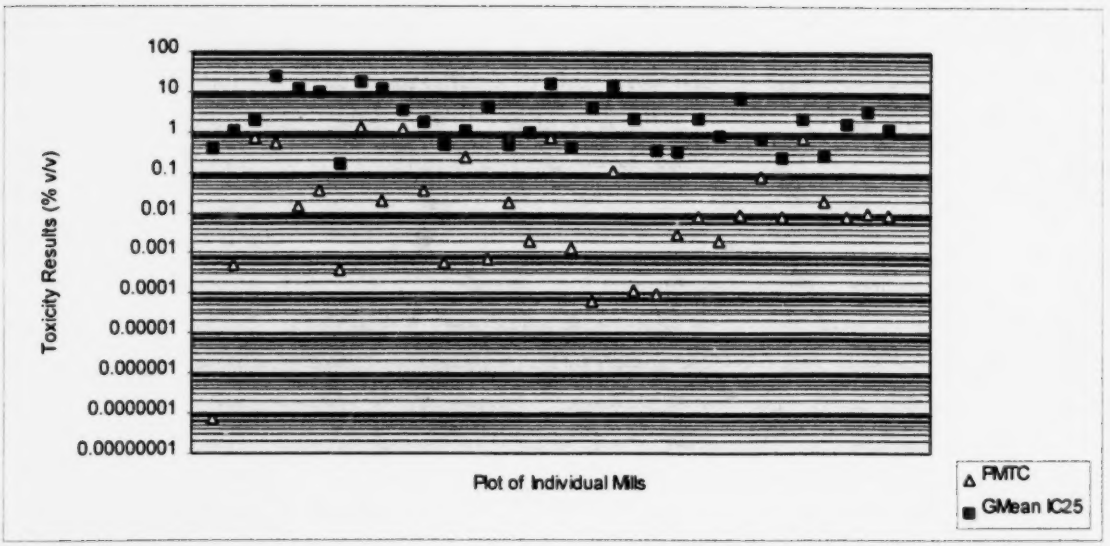


Figure 4: Comparison of *Ceriodaphnia* Reproduction Geometric Mean IC25s to PMTCs for Mills with Primary Treatment

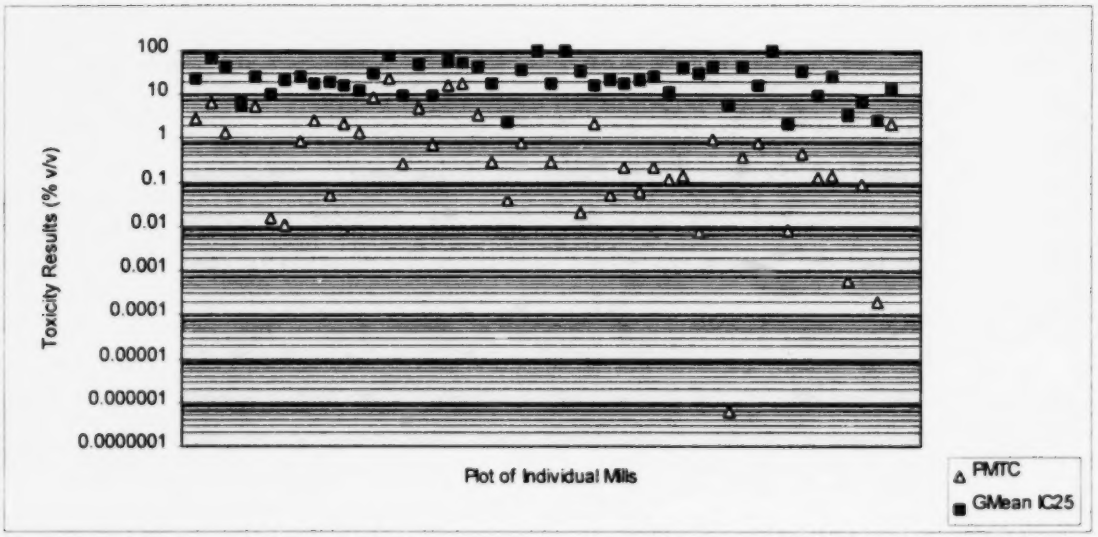


Figure 5: Comparison of *Ceriodaphnia* Reproduction Geometric Mean IC25s to PMTCs for Mills with Secondary Treatment

To get a better understanding of the influence that low numbers of test results have on the PMTC estimate, mean IC25 and PMTC values were calculated for *Ceriodaphnia* reproduction data sets from nine U.S. pulp mills with secondary effluent treatment systems. Unlike data from Cycle One, the number of *Ceriodaphnia* test results available for individual U.S. mills ranged between nine to fifteen. The gap between the mean IC25 and the PMTC was typically one order of magnitude or less for the U.S. mills. This close concordance is likely due to the fact that the U.S. mills had relatively stable effluent quality,
the *Ceriodaphnia* test results were consistently of high quality and there was a sufficient number of test results per mill.

From the analysis of the U.S. mill data, a number of conclusions can be drawn:

- a greater number of toxicity data points significantly decreases the standard deviation of mill test results, which results in PMTCs which are much closer to mean IC25 values;

- the PMTC is a fairly conservative estimate, even when calculated at the 95% confidence level;

- for mills which have generally low IC25s (eg: reproduction inhibition at effluent concentrations below 50%), the PMTC was usually close to the lowest observed IC25;

- for mills which had generally high IC25s (eg: reproduction effects only in the 50% effluent concentration or higher), the PMTC was usually significantly lower than the lowest observed IC25;

- the PMTC estimate was generally more realistic when a larger set of data was available.

Another option for evaluating potential zones of effect is to use individual IC25 responses. Sublethal toxicity tests are very useful measures for the assessment of effluent quality and the strength of these tests lies in the use of each individual test result. Each individual sublethal test result can be assessed in relation to the series of samples tested sequentially from the same effluent. This temporal information is lost in many of the other available statistics. When used this way, the IC25 itself is the important measure, not the tails of the distribution which can be highly variable depending on the number of samples involved, and the temporal variation in effluent quality.

Some concerns were raised about the use of the PMTC in the pulp mill effluent EEM program. Those concerns are grouped under 3 headings: normality of the data, sources of variation, and interactions with time and process.

The original book from which the PMTC is derived (Selected Techniques of Statistical Analysis, 1947) states that the satisfactory application of tolerance limits assumes that the successive values of the parameter in question may be considered randomly chosen from a statistical universe. The authors considered that tolerance limits may be irrelevant if the underlying distribution is too far

from normal. The decision about the adequacy of the normality assumption may be made upon the analysis of measurements of a large number of items, on theoretical considerations, or on a combination of both.

It should be pointed out that if the data in question are not normal then most of the parametric statistical procedures used for samples of this type may be invalid also.

Despite the uncertainty surrounding the question of data normality and the availability of only 4 data points per test, the original writers on the 1995 Environment Canada's guidance document intended the PMTC to provide a very rough estimate of the lowest possible toxic concentration of effluent. As with confidence intervals, the variation will tend to be high for small sample sizes.

There are two main sources of effluent variation that can influence the PMTC values: 1) variation in the response of the test system; and 2) significance changed in effluent quality due to in-mill process changes, treatment system changes or seasonal influences. Test system variability can be due to variation in organism response or variation in technique. The use of a quality control system based on the regular testing of a reference toxicant is intended to control the non-effluent sources of variation.

The original reference on "Tolerance Limits" states that assignable causes of variability be detected and eliminated so that the remaining variability may be considered random. For example, if a laboratory is performing well, then a 30-day window around the toxicity tests is probably an acceptable interval for the testing of the positive control. It may not be adequate, however, if the laboratory is not performing well. There is concern that the control of laboratory performance is based on internal systems. Such systems are clearly a start, but one needs to have confidence that the laboratory component of the "response" is properly controlled.

In Cycle One, it was apparent that a consistent quality standard for the sublethal toxicity tests was missing and consultant laboratories were allowed to control their own performances and results. For Cycle Two, an external QA program such as that provided by CAEAL or MEF or an audit program arranged by the industrial client should help. Changes recommended for Annex 1 to strengthen the QA/QC requirements will also aid in controlling laboratory sources of test variability.

An *a priori* assumption in the use of the laboratory toxicity tests was that they would be able to detect improvements in effluent quality over time with the introduction of process changes. An interaction with time might to affect the distribution of the data, thus calling into question the underlying assumption of data normality.

Such a change in test response could also give rise to different populations of data, with possibly non-homogenous variances (eg: improvement in effluent treatment from primary to secondary). The guidance should clearly state that the "before" and "after" values are not to be combined to generate a PMTC number. The two different populations must be treated separately. The data should be carefully examined to see if there is an indication of different behaviour. The sources

of toxicity and variation in a sample may change after a process alteration in a mill. Also, effluent in winter could conceivably have different toxicity properties than effluent in summer. Pooling of such data could increase variation and cause unreasonably depressed PMTC values.

3.2 Issues Related to Effluent Collection, Transport and Toxicity Test Methodology

Toxicologists from the major private testing laboratories who conducted most of the Cycle One sublethal toxicity testing provided considerable feedback. Their issues and concerns are outlined in Apendix E and were the subject of a five hour videoconference call involving representatives of the principal private consulting laboratories who conducted the EEM toxicity testing for industry during Cycle One. The EWG's views on most of these issues are outlined below.

3.2.1 Sample Collection and Transportation Issues

Sample Cooling During Transport: For some very hot primary treated effluent samples, icepacks leaked in the shipping containers during Cycle One. B.C. Environment uses frozen receiving water to cool the sample to help maintain its integrity. Although the EWG agreed that cooling the sample during transport is important, members felt that use of regular ice should be recommended over "blue ice" packs. The use of Reliance™ 22L plastic, collapsible, cube shaped containers placed in coolers and packed with regular ice for shipping of effluent is also recommended.

Need for One Versus Three Discrete Effluent Samples: The EWG agreed that guidance was needed on when one vs three effluent samples should be recommended and supported the preparation of clear guidance on this issue by Environment Canada. Members agreed that 72 hour test initiation limit requirement should remain a "must" but felt that the limit should be expressed as 3 days to provide greater flexibility.

3.2.2 Generic Methodology Issues

Improper Statistics for Specific Endpoints:
For sublethal toxicity tests requiring endpoints for survival and growth or reproduction, the EWG agreed that an LC50 calculated using quantal statistics and an IC25 calculated using quantitative statistics will be required. It was also agreed that at least one test concentration must bracket each side of the IC25 value. There were a number of Cycle One tests where no endpoint was obtained for toxic effluent samples due to an inadequate number of test concentrations. This resulted in "less than" values being reported. For Cycle Two, the EWG feels that this situation is unacceptable and a quantitative endpoint must always be determined.

Randomization of Test Containers:
Randomization is an important scientific principle but must be weighed against the risk of technician error in toxicity testing. The EWG recommends that randomization of test concentrations should be required for all EEM sublethal toxicity tests with the exception of the *Selenastrum capricornutum* microplate test where the plates should be randomly distributed throughout the incubator.

Salinity Adjustment for Marine Tests:
EWG will provide guidance on salinity adjustment for all marine tests as it does not currently exist. The following statement should be added to Annex 1: "In the conductance of all marine tests, salinity adjustment must be carried out in the manner described by Environment Canada".

Minimum Level of Reporting:
There was complete agreement within the EWG that minimum reporting requirements must be included in methods used for the EEM program.

Too Many Required Endpoints:
The EWG recommends some changes in Annex 1 in the area of endpoint reporting. Test endpoints to be retained are IC25 and 95% confidence interval, LC50 and 95% confidence interval for tests where there is a survival endpoint and an EC25 and 95% confidence interval for the salmonid embryo test.

The following endpoints should be removed from Annex 1: NOEC, LOEC, TEC, IC50 and 95% confidence interval. Hypothesis testing is the subject of greater debate and many organizations recommend against its use (ie: OECD, ISO, certain U.S. EPA programs).

3.2.3 Specific Methodology Issues

***Champia* Test:**
From Cycle One data, it is apparent that the variability between replicates was too high. The EWG recommends that specific method modifications suggested by participants of the third EWG meeting (ie: use of two male branches to ensure sufficient availability of gametes and use of a device to provide gentle agitation of test solutions) are recommended as a way to reduce the variability of the *Champia* method.

Fathead Minnow Test:
Are Growth and Survival Endpoints Equally Sensitive? More data would be necessary to judge whether or not one endpoint could be dropped. This issue should be re-examined after Cycle Two.

Possibility of Greater Flexibility With Regard to Larval Age at Test Initiation: The EWG has decided that no change should be recommended. Published U.S. EPA studies have indicated that less than 24 hours old larvae are important for test initiation. This was confirmed in a recent discussion with EPA staff at the Duluth laboratory.
Need to Increase Minimum Control Growth Validity Criteria: The consensus was that no change is warranted at this time.

***Selenastrum* Test:**
Absorbance Methods: For pulp mill effluents, the EWG considers absorbance-derived endpoints to be unacceptable. Electronic particle or manual counting are recommended.

Quality of Test Organisms: The EWG agreed that the method should be changed to state that renewal of the liquid algal stock culture using a solid medium culture must be conducted every two months.

Growth Curves: The EWG recommends that growth curves be generated only at one initial cell concentration (ie: 10 000 cells per mL) on a frequency of at least two times per year. It is recommended that the preparation of a growth curve should be initiated when there is a noticeable change in colour in the stock culture (eg: yellowing of culture). It is also recommended that algal testing should not be conducted on the first liquid culture following initiation of a new culture from a solid medium.

Minimum Holding Time for Algal Solid Culture: The EWG recommends that the algal solid culture should be replaced every 6 months but must be replaced once a year.

Washing of Glassware: The EWG feels that there is no point in pre-washing disposable glassware and this should be made clear in the method document.

Preparation of Enrichment Medium: The EWG recommends to keep the test duration at 72 hours and that the enrichment medium remain a required part of the test method.

Preparation of Algal Inoculum: The EWG recommends that the test method be changed to eliminate the second centrifuge step of the algal washing procedure. However, members feel that resuspension in $NaHCO_3$ solution is still required.

Preparation of Test Solution: The EWG recommends no change to the method regarding pH adjustment of the sample.

Procedure for Incubation: The EWG recommends the use of microplates with round bottom wells and suggest that the limitations of microplates with flat bottom wells (ie: difficulty in resuspending cells and pH shift over greater than 2 units) be stated in the revised test method.

Quality Control Microplate and Validity Criterion: The EWG recognizes that the quality control microplate was instituted to address the problem of volatile substances. The EWG is considering other solutions to the volatile substances issue which could lead to the elimination of the quality control microplate. Possibilities include: 1) checking for a gradient effect in the control wells and 2) checking if there is growth inhibition in the controls of the test microplate. When a volatile substance problem is identified, the test would have to be repeated using one microplate per concentration or a switch could be made to the U.S. EPA flask method. This issue is still under discussion.

Test Endpoint Calculations: The EWG recommends to stay with the cell yield as the means for calculating inhibition. Growth rate as an endpoint is not recommended since it requires measurements at various times during the test, which is largely impractical for a routine test method.

PH Drift Validity Criterion: The EWG recommends that the method be changed to drop pH drift as a validity criterion as long as a check for pH drift is made each time a new growth curve is prepared. Guidance is needed on how to check for pH drift (ie: need to pool wells to obtain enough volume for a pH measurement).

***Ceriodaphnia* Test:**

Use of Fisher's Exact Test to Identify Significant Mortality: The EWG deferred on their thoughts related to this issue since it does not seem to happen very often. Guidance from EC will be provided later.

ICp Derivation: The concentrations chosen must bracket the IC25 endpoint. The issue of what to do about adult female mortality in the statistical calculation has been dealt with by the EWG decision to calculate LC50 using quantal statistics and calculating IC25 for reproduction using quantitative statistics. Further guidance will be provided in the revised method.

Endpoints and Calculations: The EWG recommends the following for the EEM program:

- must start with 100% or highest effluent concentration possible if survival is an endpoint;
- must have test concentrations which bracket each side of the IC25 (with the exception of greater than 100% results);
- must calculate an IC25 by quantitative statistics while EC25 and LC50 must be calculated using quantal statistics;
- must generate a quantitative estimate of LC50, IC25 or EC25 (ie: no "less than" values acceptable).

Limited Age Window (8h): The EWG agreed to extend the age window from 8 to 12 hours and recommends this change be made in the method revisions. However, members agreed that the criterion for "neonates of less than 24 hours old" must stay as outlined in the test method.

Test Duration: The EWG agreed that the test will terminate when 60 % of the adult females in the control have had greater than 3 broods and an average of at least 15 young per female have been produced within 8 days.

Salmonid Embryo Test:

There were a number of issues identified during Cycle One by consulting laboratory staff conducting this test (eg: suitability of survival as the principal test endpoint, availability of gametes from hatcheries, question of whether to increase test temperature, large volume of effluent required, use of alternative test containers, use of an egg incubation basket, etc). These questions and issues were resolved through a method improvement test program conducted by Environment Canada during 1996. During the fall of 1996, Environment Canada prepared a draft revised method with suggested changes to address the issues identified. Representatives from PAPRICAN, government and private consulting laboratories provided useful comments which lead to the finalized version of the salmonid embryo test (second edition). The revised method is currently in press and will be distributed in June 1997.

3.3 Available Alternative Methodologies

3.3.1 Cycle One Tests

It was a consensus opinion of the EWG that the EEM Cycle Two program "stay the course" on the test battery approach using a fish, invertebrate and algae toxicity test that was taken in Cycle One. EWG members felt that by not changing the basic core sublethal testing groups from Cycle One, the continuity into Cycle Two would remain despite any changes that may arise from adding alternative or deleting specific tests from any of the core groups.

Of the original sublethal methods from Cycle One, there was consensus that two methods had not performed (ie: lack of standardized method, lack of sensitivity and/or no use of the test procedure, see Section 3.1.2 for details). As a result, there was consensus that two testing methods should be dropped. The two methods were the 1) *Lemna minor* duckweed plant test and 2) the sheepshead minnow (*Cyrindon variegatus*) a marine embryo-larval fish toxicity test.

3.3.2 Method Screening Criteria

The decision process to add alternative toxicity methods focused on the three criteria for selecting monitoring variables plus the eight generic principles for the EEM program as cited on page 3 of EPS 1/RM/18, and a list of five additional "must" criteria that the EWG agreed should be used to screen potential alternative or additional sublethal methods for Cycle Two. These five criteria were: 1) a published test procedure; 2) must have already been used in effluent testing; 3) uses an organism of ecological/local relevance; 4) endpoint must be ecologically relevant; and 5) method must have been validated through inter-laboratory round robin testing.

These five criteria were arranged in a table format and each of the alternative or additional sublethal methods considered were evaluated against the five criteria. Prior to the group discussion on each of the candidate tests, details on the methodology were distributed to the members for review. The results of this process can be found in Table 8. In summary, four of the methods met the five criteria and became methods with "potential" to be included into the suite of sublethal tests for Cycle Two. These included the two freshwater sediment toxicity methods using *Chironomus spp.* and *Hyalella sp.*, the marine amphipod sediment method and the topsmelt survival and growth method for effluent monitoring. There was consensus that the topsmelt method be accepted as a suitable replacement for the sheepshead minnow and as a logical alternative for use in testing of effluents discharged from marine mills in British Columbia. Two other methods, the macro-algal reproduction test using *Ceramium* and salmonid embryo/alevin test met all the major benchmarks with exception of inter-laboratory round robin testing criterion. The Microtox Chronic test failed to meet the inter-laboratory round-robin and ecological relevance criteria. The EWG heard from various members about plans to validate the Microtox Chronic and the salmonid embryo/alevin methods. The final test method considered was a genetic toxicology procedure which measures chromosome damage in fish red blood cells using flow cytometry (CDC test). However, this procedure did not meet two of the five minimum criteria (ie: available standardized procedure and inter-laboratory validation).

Table 8: Criteria for Acceptance of Alternative Test Methodology

	Published Test Procedure	Already Used with Effluents	Organism Ecological Relevance	Endpoint Ecological Relevance	Round Robin Validation
Ceramium Sexual Reproduction	√	√	√	√	No
Chironomus spp (Sediment)	√	NA	√	√	√
Hyalella sp (Sediment)	√	NA	√	√	√
Microtox Chronic	√	√	No	√	No
Topsmelt, *Atherinops affinis*	√	√	√	√	√
Salmonid Embryo/Alevin	√	√	√	√	No
Marine Amphipod (Sediment)	√	NA	√	√	√
Fish Chromosome Damage	No	√	√	?	No

√ = criterion successfully achieved
NA = not applicable
? = no consensus among EWG members

Rationale for whether or not a method was accepted or rejected was solely based on presentations, documented literature and the criteria table established by the EWG.

Tests Failing to Meet the Acceptance Criteria

The genetic toxicity method, chromosome damage using flow cytometry (CDC), tabled by Dr. Easton failed to meet the round robin validation and published standard procedure criteria while there was no consensus among EWG members on the ecological relevance of the endpoint. The Microtox Chronic method by Azur Environmental was rejected on the basis that there were not any inter-laboratory round robin testing using pulp and paper effluents and concerns about the ecological significance of the test organism. The EWG heard plans for an inter-laboratory study planned for the Spring of 1997 with seven laboratories to test variability with pulp mill effluents.

Ceramium, a marine macro-algae, has potential as a replacement for *Champia* as a test species. However, this fertilization method did not meet the round robin validation criterion. This method should be a candidate for future EEM cycles as an alternative to the *Champia* test, especially since *Ceramium* is a temperate algal species found in inter-tidal areas off the Pacific and Atlantic coasts of Canada. Pulp mill effluent validation testing for the salmonid embryo/alevin test is planned for the near future by Environment Canada.

New Tests

The EWG considered the following factors in assessing whether new methods should be recommended for possible use in Cycle Two: 1) meeting the original criteria found in EPS/1/RM/18; 2) the five criteria bench marks established by the EWG; 3) reasonable ability of Canadian private sector laboratories to offer testing services for new test; and 4) convincing justification for recommending the inclusion of the test for core or site-specific use in Cycle Two.

The topsmelt marine larval test was tabled and accepted as the replacement for the sheepshead minnow and an alternative to the inland silverside method. One of the main reasons for its acceptance was that the marine fish test options from Cycle One involved sub-tropical species not indigenous to Canadian marine waters, while the topsmelt can be found in waters off Vancouver Island. As well, the topsmelt method is currently used by the States of Washington, Oregon and California by both industry (including the pulp and paper sector) and State and Federal Environmental agencies as a replacement for the inland silverside test. However, the decision to accept the topsmelt test does not preclude the Pacific and Atlantic mills from using the inland silverside method. The EWG recommended that the local EEM authorization committees on both coasts be the body who decides which of the two marine fish method options would be the best suited for local site specific conditions.

The freshwater and marine sediment toxicity testing methods met the minimum acceptance criteria established by the EWG and are available for appropriate use. Following a presentation on the sediment methods and hearing about the benefits to be gained by appropriate use (eg: assist in understanding whether benthic impacts are due to historical impacts, pulp mill impacts or other sources of contamination), the EWG felt that these methods could be used in specific situations to supplement Cycle Two monitoring. However, it was also recommended that the benthic community EWG should also consider the use of sediment toxicity tests in their discussions of decision tree elements and triggers. The EWG agreed that for sediment toxicity assessment, the freshwater *Hyalella* or *Chironomid* spp. tests and the marine multi-species amphipod sediment method were good candidates for appropriate use within the EEM program.
Their use should be governed by site-specific factors (eg: must have sediment depositional zones at near field sites, complex multiple discharge situation, gradient of effect detected in benthic survey but uncertainty as to the source of effects, etc).

The EWG agreed that if a site requires further investigation or examination of historical contamination (ie: toxicity associated with sediments), then sediment toxicity tests could be useful. However, use of the sediment toxicity tests will have to be discussed further with representatives of the EWG on Benthic Community and members of the TMC.

3.4 Linking Toxicity Test Results to Field Effects - Can it / Should it be Done?

The EWG has had considerable debate on this issue, covering questions on the original objectives of the sublethal toxicity tests, assessing the predictive capacity of the current tests in Cycle One, and discussing the goals of the toxicity tests as we move forward into Cycle Two.

The EWG agreed that it is too early to interpret whether sublethal tests are predictive of field effects in EEM, as there are only four data points per test at each mill from Cycle One.

The EWG agreed that results of sublethal tests are useful to monitor effluent quality over time and to assess the relative contributions in areas that receive effluents from multiple sources. We also agreed that the sublethal tests make valuable contributions to the EEM program in that they contribute to the "weight-of-evidence" approach. Some EWG members believe that the use of toxicity tests was never meant to predict field effects within the EEM program, while others believe that toxicity tests implicitly involve an intent to predict field effects.

The EWG recognizes that there is variability and uncertainty in the field responses, and that making definite conclusions on whether effluent is causally involved is difficult. However, information on the effluents themselves is important to identify the potential relationship to make these types of judgment.

The diversity of biological effects that wild organisms can demonstrate with effluent contaminant exposure cannot be captured by a few laboratory toxicity tests with simple endpoints. On the other hand, if types of organisms and endpoints used in the test are relevant to what we are trying to protect (ie: fish and fish habitat) then there will be increased confidence in suggested links, particularly where these endpoints affect the health of important aquatic organisms.

Assessing Cycle One Sublethal Toxicity Data for Links to Field Effects

The EWG had some reservations about the assumptions necessary to allow the consultants to classify the toxicity data. Generalizations using wide criteria were necessary to classify the test results. Toxicity test results were classified into broad "Effect" or "No Effect" categories, then compared to field data that has been categorized similarly, to arrive at a "Yes" or "No" decision on whether there was or was not correlation between laboratory and field. These attempts were useful and provided valuable data from those working closest with Cycle One field data and laboratory tests. But it should be stressed that the correlations and conclusions are preliminary and the assessments were broad and affected by personal judgment. With these caveats in mind, the following section presents a summary of the views of knowledgeable individuals regarding the predictive capacity of the sublethal tests in Cycle One (ie: people who have attempted to interpret whether a relationship exists between lab and field data from Cycle One).

Presentation by Stan Megraw (DOMTAR)

Stan Megraw, at the EWG request, provided information on the potential relationship between laboratory sublethal effects and observed field effects from his in-depth knowledge of Cycle One studies conducted at seven DOMTAR mills. According to Stan, evidence of field effects was found only near two of the mills studied. For one of the mills, he indicated that although numbers of organisms and diversity was high, increased levels of total carbon at stations near the outfall, coupled with low average richness and the presence of pollution tolerant organisms, suggested an effect of the mill. For the second mill the abundance and richness was much lower in the

exposure zone. At this mill, there was also depressed oxygen levels in the river until secondary treatment was installed in 1996. There was significant sublethal toxicity response for both these mills before and after secondary treatment (although significantly reduced after installation of secondary treatment). For the second mill, a special study was conducted when ambient water from the effluent plume was collected for toxicity testing. The exposed *Ceriodaphnia* did not confirm the response seen from the effluent toxicity test using laboratory water. It was Stan's opinion that laboratory toxicity tests were not predictive of sublethal effects in the receiving environment.

Presentation by Roy Parker (EC)

Roy Parker gave a presentation on the relationship among the effluent sublethal toxicity, adult fish survey (AFS) and invertebrate community survey (ICS) elements for the 17 EEM studies conducted in Atlantic Region. Roy used the term "observed differences" and stayed away from the term "effects". Roy also indicated that the following qualifiers should be clearly understood:

- this was a qualitative comparison;
- there were many weaknesses in the AFS component;
- fish exposure to effluent was confirmed at only 3 of the 17 mills; and
- there were other sources of contamination at several sites.

A "difference" was counted for his presentation when a response in one or more species or parameters was observed. In a comparison of AFS results to fish sublethal toxicity results, the toxicity test predicted correctly that there should be effects in adult fish in 5 of 9 marine mills and 2 of 3 freshwater mill sample sites. Also, the fish sublethal toxicity test correctly predicted that there should be no effects of fish in 2 of 3 marine mill and 1 of 1 freshwater mill sample sites. In a comparison of the ICS results to invertebrate sublethal toxicity tests, the toxicity tests correctly predicted that there should be effects on the benthos in 12 of 12 studies and correctly predicted that there should be no effects on the benthos in 1 of 4 studies. However, there were 4 out of the 17 studies where the toxicity tests predicted differences where none were detected.

Roy's principal conclusions were that:

- the sublethal toxicity tests with invertebrates were good predictors of benthic invertebrate communities differences seen in the local receiving environments;
- the results of sublethal toxicity tests with early life stages of fish did not relate as well to the AFS perhaps as a result of the significant problems in catching the minimum number of fish and confirming exposure to the mill effluent; and
- for the freshwater sublethal fish tests, there was a stronger relationship between toxicity and fish health indicators but the data set was small (4 mills).

Paper by Patti Orr (BEAK)

The EWG reviewed a paper from Beak Consultants that was presented by P. Orr at the 83rd Annual Meeting of the CPPA Technical Section. Beak conducted field and laboratory studies at 25 mills from across Canada. The majority of the receiving environments were rivers. Fifteen of the twenty five mills had secondary treatment in place at the time of the EEM study.

The authors concluded from their data that toxic effects on biota (ie: fish populations or benthic invertebrate populations) in the receiving environment were predicted at 15 mills (60%) under typical low effluent dilution conditions based on sublethal toxicity tests results and effluent dispersion conditions. In all of those cases where effects were predicted, effects were also observed in the receiving environment. It was acknowledged that the effects on biota in the receiving environment could not, at this stage, be attributed to direct toxic effects of the effluents, as opposed to indirect effects such as nutrient enrichment or avoidance responses. At 6 mills (25%), toxic effects were not predicted yet effects were observed in the field (false negatives). The authors speculated that those effects may have been caused by influences other than toxicity. The authors' point out that there was an apparently low risk of false positive results since, in this review of 25 mill studies, the sublethal tests did not predict effects where none were observed in the field.

EWG members observed that the statistical analysis of the possible relationships has yet to be undertaken. Also, field effects were observed at 21 out of 25 mills, which means that the tendency of the tests to falsely predict a positive field effect has been based on data from only 4 mills. It was noted that Beak used the IC25 rather than the PMTC to predict zones of potential effects in their studies.

Requirements to Aid the Development of Predictive Sublethal Tests

The EWG felt that if sublethal tests with more predictive ability were to be developed, several pieces of information were necessary. The following questions and suggested answers would aid in the development of meaningful and predictive laboratory sublethal tests.

What are the measurable field effects of pulp and paper mill effluent that we are trying to predict? From EEM field data, and from scientific studies of pulp and paper mill effluents, defining the important biological effects observed in the field would aid in the development of laboratory tests predictive of these effects.

What are the biological mechanisms behind the observed field effects? If we know the mechanism, we can design a laboratory toxicity test with better potential predictive capability based on a mechanistic approach.

What is it in the effluent that causes the effect? Knowledge of the chemical(s) or classes of chemicals causing effects would allow development of specific predictive laboratory tests.

Potential Impediments to Using These Sublethal Tests as Predictive Tools

The EWG felt there were many uncontrollable and unknown factors that may prevent the development of improved predictive sublethal tests or may confound predictions in many environments.

These factors include:

- Methodological Issues: Toxicity tests often use lab water rather than receiving water, as the dilution water in exposures. Use of up-stream or reference site receiving water for laboratory test controls and test concentration dilutions would increase the predictive power;

- Abiotic Factors: Environmental variables such as UV light, pH and DOC may have a profound influence on effects manifested in field organisms compared to the laboratory and would have to be considered;

- Biotic Factors: Difference in species sensitivity can translate into disagreement between laboratory predictions and observed field effects;

- Confounding Factors: Environmental situations are often complex, with multiple point and non-point contributions that influence observed field effects but not laboratory effluent toxicity tests. Toxicity tests conducted on ambient water samples collected along the effluent plume gradient might be one approach to this problem.

The Path Forward

The EWG felt that the future for toxicity tests is to investigate and move towards predictability in the field. Assessment of potential predictive ability should be made with the additional data generated by future EEM cycles.

At three out of five industry/government EEM workshops (Fredericton, Toronto, Montreal), representatives felt that an attempt to link laboratory toxicity test results with findings in the field in the future was needed.

A goal of research outside the EEM program is to improve toxicity tests and to assess their predictive ability. Tests within the EEM should be assessed in subsequent cycles when more laboratory and field data have been generated.

EWG members have concluded that the most effective approach to interpreting sublethal toxicity test data is to use good scientific judgement and to consider this laboratory data with all site-specific EEM information following a weight-of-evidence approach.

Although there was apparent concordance among observations in the field and sublethal toxicity tests measured in the laboratory with effluent samples, EWG members expressed uncertainty about the methodologies employed to make these links. Members have concluded that these relationships be re-examined further in subsequent cycles.

The EWG felt that future EEM program cycles should be designed to assess linkages between sublethal toxicity test results and field effects as methodologies are improved through research and that future research address the questions of predictive capacity of current tests and focuses on the development of predictive laboratory tests.

4.0 Conclusions and Recommendations

4.1 Conclusions

Overall, Cycle One sublethal toxicity testing was successfully completed and it should remain part of the core program for Cycle Two. Even though secondary treatment was designed to eliminate acute lethality and reduce suspended solids and BOD, it also significantly improved effluent quality with respect to sublethal toxicity. The EWG concluded that some refinement to specific test methods and Annex 1 changes to strengthen the quality assurance aspects of EEM sublethal toxicity testing were needed. As the sheepshead minnow test was not used in Cycle One, the EWG concluded that this test option should be replaced by the topsmelt survival and growth test. The topsmelt is a marine fish species found off the Canadian west coast and is a test organism used in an accepted USEPA test procedure. EWG members also concluded that the *Lemna minor* test should be removed as a test option leaving the *Selenastrum* test as the sole freshwater plant test. The EWG agreed that all other Cycle One sublethal toxicity tests should be retained in Cycle Two.

The EWG made a number of specific recommendations for resolution issues related to effluent collection and test methodologies (see Section 3.2).

Although a concerted attempt was made to establish a single consensus position on the frequency of sublethal toxicity testing for Cycle Two, the EWG has prepared two options and supporting rationale for consideration by members of the TMC: 1) two testing periods per year (ie: total of 6 testing periods over a typical 3 year cycle); or 2) four testing periods per year (ie: total of 12 testing periods over a typical 3 year cycle). Rationale supporting the two options for frequency of testing is outlined in Section 3.1.4. However, EWG members agreed that the frequency of testing should be uniform across all mills for Cycle Two.

EWG members have concluded that the most effective approach to interpreting sublethal toxicity test data is to use good scientific judgement and to consider this laboratory data with all site-specific EEM information following a weight-of-evidence approach.

Although there was some apparent concordance among observations in the field and sublethal toxicity tests measured in the laboratory, EWG members expressed uncertainty about the

methodologies employed to make these links. Members have concluded that these relationships should be re-examined further in subsequent cycles.

4.2 Recommendations

Recommendations of the Expert Working Group on Laboratory Toxicology have been separated into two categories: 1) recommended changes to Annex 1; 2) other recommendations which include changes to Environment Canada interpretative guidance document and recommended laboratory research and method validation studies for future EEM cycles.

4.2.1 Recommended Changes to Annex 1 for Laboratory Toxicology Component

- Retain the current battery of sublethal toxicity tests outlined in Table 7 of Annex 1 with the exception of two tests: the *Lemna minor* test and the sheepshead minnow survival and growth test;

- Remove the *Lemna minor* duckweed growth inhibition test from Table 7 leaving the *Selenastrum* growth inhibition test as the sole freshwater plant sublethal toxicity test;

- Remove the sheepshead minnow test from Table 7 and as a replacement test, cite the USEPA 1995 marine fish method for survival and growth using topsmelt (*Atherinops affinis*);

- Modify Table 2 of Annex 1 to increase the number of testing periods for sublethal toxicity testing to either 6 or 12 during a typical three year cycle. The rationale behind each of the choices is discussed in Sections 3.1.4 and 4.1. Regardless of the testing frequency chosen, ensure the testing periods are spread out over the EEM cycle;

- The specific objectives for all components of the EEM program including effluent sublethal toxicity testing should be stated in Section 2 of Annex 1. The statements and rationale below reflect a final consensus reached by all EWG members at the last working group meeting. The following three objective statements (and the supporting paragraphs) are recommended for inclusion in Annex 1:

    - To compare effluent quality between mill types and to measure changes in effluent quality as a result of effluent treatment and process changes;

    - To determine the relative contributions of multiple discharges; and

    - To assess the potential impact of an effluent on the receiving environment.

"The EEM program is designed to assess the adequacy of the national effluent regulation in protecting fish, fish habitat and the fisheries resource. This is done by conducting fish surveys,

benthic community surveys, laboratory toxicology and measurement of dioxins and tainting in fish. Sublethal toxicity measurements on the effluents themselves are essential to determine the biological effects of these effluents, now that regulatory requirements for BOD, suspended solids and acute lethality have been achieved. The response of aquatic organisms exposed to effluents under controlled conditions can be used to quantify and compare the toxicological characteristics of mill effluents over time. Similarly, these tests could be used to compare potential contributions of discharges from multiple sources.

Within the current limits of our understanding of the relationship between biological responses measured in the laboratory and those measured in the field, laboratory toxicological measurements may provide an indication of effects in the environment. For example, a general improvement in effluent quality shown by toxicity data suggests that we should expect an improvement in the aquatic environment. If the results of future research show that the toxicity tests have the ability to predict effects on wild organisms, then these tests will be helpful in assessing the potential impact of pulp and paper mill effluents".

- Modify Table 2 of Annex 1 to include a specific time frame for sublethal toxicity data reporting by industry and data review response by government (eg: data from each sublethal toxicity testing period must be submitted to the Regional Authorization Officer within 60 days of test completion. Any difficulties with the submitted data must be communicated by the Regional Authorization Officer or designated representative to mill staff within 60 days);

- Two freshwater sediment toxicity methods (ie: *Chironomus spp.* survival and growth and *Hyalella azteca* survival and growth tests) and the marine multi-species amphipod sediment toxicity method met the minimum acceptance criteria established by the EWG. Table 8 of Annex 1 should be up-dated to reflect these methodologies;

- A statement that laboratories with CAEAL, MEF or an equivalent level of accreditation for sublethal toxicity testing should be hired by mills to conduct required testing for Cycle Two should be added to Section 5 of Annex 1;

- Given that there was full support for the establishment of a minimum level of reporting for all EEM sublethal toxicity tests (and that this should be stated in Section 8.4 of Annex 1), the EWG recommends that the Cycle One QA/QC checklists be revised in a manner which will be useful to mill staff and government EEM staff in the Regions;

- Statement should be added to Section 8.1 of Annex 1 that effluent sublethal toxicity tests must be initiated within 3 days of sample collection and that a new effluent sample will have to be collected if the limit has not been achieved;

- To address QA/QC problems identified in the Cycle One data for sublethal toxicity testing, the following statements should be added to Section 5 of Annex 1:

- If any toxicity test method validity criterion has not been met, then testing of a new effluent sample is required;

- Reporting of "less than" values as a test endpoint (eg: IC25 < 10%) are not acceptable as this is an indication that there were insufficient test concentrations. All test endpoints must be bracketed by at least one test concentration with the exception of "greater than 100%" results;
- Reference toxicant tests must be conducted within 30 days of the effluent test and this test must be performed under the same experimental conditions as those stipulated for the effluent sample in the EEM sublethal toxicity test reference method.

- The following changes should be made to Table 7 of Annex 1:

  - Add a superscript (ie: ^{2}) denoting a second footnote on salinity adjustment procedure behind the test species name for each marine toxicity test. The footnote should state that the effluent salinity adjustment procedure recommended by Environment Canada must be followed;

  - Under Reporting Requirements Code "a": modify line to read "For effluent, LC50, 95% confidence limits and indication of the quantal statistic employed";

  - Under Reporting Requirement Code "b": modify the first sentence to read "For effluent, IC25, 95% confidence limits and indication of quantitative statistic employed and EC25, 95% confidence limits and indication of quantal statistic employed (ie: EC25 is applicable to salmonid embryo test only). Discontinue reporting of NOEC/LOEC/TEC and IC50.

4.2.2 Other Recommendations

The following recommendations should be considered as advise to the TMC but are not changes proposed for Annex 1:

- EC should consider the specific recommended methodology changes outlined in Section 3.2 of this report when preparing the official amendments to their sublethal toxicity test methods;

- As submission of sublethal toxicity results and QA/QC information in a consistent and common electronic format would be a cost-effective and time-efficient approach beneficial to both mill and government staff, the EWG recommends that a demonstration pilot project be initiated to develop an electronic data reporting system prior to Cycle Two data entry;

- Decision criteria for the granting of a reduced frequency of sublethal toxicity testing should be developed following the completion of Cycle Two. The EWG felt that this would be a positive incentive for mills with high quality effluent discharges;

- The specific method modifications to the *Champia* reproduction test suggested by participants of the third EWG meeting (ie: use of two male branches to ensure sufficient availability of gametes and use of a device to provide gentle agitation of test solutions) are recommended as a way to reduce the variability of this test method;

- Future EEM program cycles should be designed to assess linkages between sublethal toxicity test results and field effects as methodologies are improved through research;

- Some EWG members requested that this report be published in the DFO series: Technical Reports on Fisheries and Aquatic Sciences).

- Changes proposed to EC guidance for interpretation of sublethal toxicity data are as follows:

    - Add the three sublethal toxicity objectives statements (and two supporting paragraphs) outlined in Section 4.2.1 to the introduction of the EC EEM guidance document, after wording has been finalized by the TMC and agreed to by the Steering Committee;

    - Modify the guidance to describe three approaches for estimation of potential zones of effect from sublethal toxicity data: 1) using lowest IC25 value; 2) the geometric mean of the IC25s; or 3) the Predicted Minimum Toxic Concentration (PMTC) statistic;

    - Ensure that description of the PMTC estimation approach is modified to point out some of the limitations of this statistical approach (see Section 3.1.6 of EWG report).

- Future laboratory methodology research should address the question of predictive capability of the current tests and focus on the development of predictive laboratory tests;

- Inter-laboratory studies should be conducted to assess the variability of three possible alternative sublethal toxicity tests; 1) the marine macro-algae reproduction test with *Ceramium*; 2) the salmonid embryo/alevin test; and 3) Microtox Chronic test.

Appendix A

Terms of Reference for the EEM Expert Working Group on Laboratory Toxicology

Terms of Reference

EEM Expert Working Group on Laboratory Toxicology

Purpose:

With the completion of Cycle One monitoring requirements under the pulp and paper EEM program, there is a need to examine the results of the laboratory toxicology testing and suggest adjustments to the requirements to improve the quality and value of the program. The EEM Technical Management Committee has established this working group to address this need. The working group will assess the data, examine methodology issues and provide recommendations for Cycle Two laboratory toxicology requirements to the Technical Management Committee.

Operating Principles:

The work of the Expert Working Group will be guided by the following principles:

- basis of the group's recommendations will be driven by Cycle One Regional evaluations;
- a nationally consistent approach will be respected while allowing for flexibility in addressing site-specific issues;
- recommendations will follow the guiding principles as outlined on page 1 of Annex 1;
- the EEM program is a mandatory monitoring element of the 1992 Pulp & Paper Effluent Regulations which utilizes standardized, scientifically-defensible monitoring tools to assess the effectiveness of the national regulation on a site-specific basis.

Specific Objectives:

- review the sublethal toxicity data generated during the first cycle of EEM;
- identify any specific difficulties and make recommendations related to effluent collection protocol and shipment to test laboratories;
- identify any specific concerns and make recommendations related to the toxicity test methodologies used during EEM Cycle One;
- discuss frequency of sublethal toxicity testing;
- comment on the ability of the sublethal toxicity data to detect changes in effluent quality following improvements in effluent treatment;
- discuss current guidance on interpreting EEM sublethal toxicity data;
- discuss the possibility of introducing alternative tests and criteria for new test acceptance;
- discuss whether general conclusions can be made with regards to the relationship between the laboratory sublethal toxicity data and observed field effects; and
- prepare a report which summarizes working group discussions on the above specific objectives and makes recommendations to improve the quality and value of data to be generated during EEM Cycle Two.

Membership:

Pierre Martel	PAPRICAN, Pointe Claire, Quebec (Co-Chair)
Richard Scroggins	DOE/ETC, Ottawa, Ontario (Co-Chair)
Kenneth Doe	DOE, Dartmouth, Nova Scotia
Barry Firth	Weyerhaeuser, Tacoma, Washington
Katsuji Haya	DFO, St. Andrews, New Brunswick
Lyle Lockhart	DFO, Winnipeg, Manitoba
Valerie Naish	Domtar, Ste-Anne de Bellevue, Quebec
Joanne Parrott	DOE/NWRI, Burlington, Ontario
Jerry Payne	DFO, St. John's, Newfoundland
Danielle Rodrigue	DOE/ETC, Ottawa, Ontario
Jim Sherry	DOE/NWRI, Burlington, Ontario
Donald St-Laurent	DOE, Montreal, Quebec
Michel Taché	DOE/HQ, Hull, Quebec
Graham van Aggelen	DOE, Vancouver, British Columbia
Gary Westlake	MOE&E, Rexdale, Ontario

This working group will participate in meetings (by teleconference or in person) on a regular basis for the duration of its mandate. Additional outside experts in sublethal toxicity testing (eg: Don McLeay, John Sprague, Christian Blaise, Kristin Day, Glenn Thursby, representatives from various Canadian consulting laboratories, etc.) may be consulted for specific technical expertise when needed.

Time Period:

July 96 to March 97

Deliverable:

A report summarizing the EWG's recommendations to the EEM Technical Management Committee by April 1, 1997.

Appendix B

Table B.1: ***Results of Sublethal Marine Tests for Cycle One EEM (Menidia, Echinoderm, Champia)***

Table B.2: ***Results of Sublethal Freshwater Tests for Cycle One EEM (Ceriodaphnia, Selenastrum, Lemna)***

Table B.3: ***Results of Sublethal Freshwater Tests for Cycle One EEM (Fathead minnow, Oncorhynchus)***

TABLE B.1: RESULTS OF SUBLETHAL MARINE TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Menidia beryllina* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Menidia beryllina* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	Echinoderm Fertilization IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Champia parvula* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Pacific & Yukon																				
PY-M-01	~	>65.9[a]	>67.9[a]	>67.7[a]	~	~	>65.9[a]	>67.9[a]	>67.7[a]	58.5 (n=3)	0.5[eb] (0-1.6)	10.6[eb] (9.1-11.3)	<4.2[eb]	14.9[eb] (14.1-15.6)	$3.6x10^{-2}$ (n=3)	~	4.9[a] (0.3-29.3)	<4.2[a]	5.3[a] (1.2-5.4)	1.77 (n=3)
PY-M-06	>67.9[a]	>65.5[a]	>66.7[a]	>68.2[a]	~	>67.9[a]	>65.5[a]	>66.7[a]	>68.2[a]	60.7	6.5[bs] (5.4-7.5)	35.8[bs] (33.6-38.4)	<4.2[eb]	>66.7[eb]	0.013	~	~	<4.4[a]	4.5[a] (1.9-8.5)	~
PY-M-07	~	>68.5[a]	>68.2[a]	>69.9[a]	~	~	>68.5[a]	>68.2[a]	>69.9[a]	61.7 (n=3)	18.9[bs] (15.7-21.7)	6.2[bs] (5.1-7.4)	1.5[eb] (1.2-1.9)	<4.3[eb]	0.02	~	6.3[a] (-5.9-19.0)	<4.1[a]	5.5[a] (2.2-8.0)	0.83 (n=3)
PY-M-11	~	~	~	~	~	52.6[a] (24.5-59.3)	96.2[a] (47.3-96.5)	>100[a]	>100[a]	15.52	11.2[eb] (10.5-12)	0.9[eb] (0.8-0.9)	9.5[eb] (9.0-9.9)	0.7[eb] (0.6-0.9)	0.001	<4.4[a]	<4.4[a]	12.4[a] (8.5-17.0)	<4.4[a]	0.35
PY-M-12	~	~	~	~	~	>67.9[a]	>66.2[a]	>67.9[a]	>67.7[a]	63.13	4.5[bs] (2.3-5.7)	0.1[eb] (0.1-0.2)	<4.2[eb]	6.3[eb] (5.9-6.6)	$5.1x10^{-5}$	16.5[a] (-16.7-24.0)	5.3[a] (-4.3-7.6)	<4.2[a]	11.4[a] (5.2-21.9)	0.26
PY-M-16	20.4[a] (16.4-23)	19.5[a] (18-21)	16.4[a] (9.8-20.2)	>67.7[a]	~	>17.9[a]	>16.5[a]	>16.4[a]	>67.7[a]	0.56	<0.1[bs]	~	<4.1[eb]	<4.4[eb]	$1.6x10^{-6}$ (n=3)	<1.0[a]	<1.0[a]	<4.1[a]	<4.2[a]	0.02
PY-M-17	>70.9[a]	>69.0[a]	>65.8[a]	>65.4[a]	~	>70.9[a]	>69.0[a]	>65.8[a]	>65.4[a]	55.02	~	~	13.3[eb] (11.9-15.5)	9.3[eb] (1.0-11)	~	17.6[a] (-18.3-23.6)	6.4[a] (-5.3-8.7)	13.2[a] (2.9-58.9)	>63.4[a]	0.1
PY-M-18	>63.8[a]	>67.1[a]	>65.8[a]	>64.6[a]	~	>63.8[a]	>67.1[a]	>65.8[a]	>64.6[a]	58.03	>62.5[bs]	48.1[bs] (43.4-52.1)	10.4[eb] (9.2-11.8)	15.7[eb] (14.0-18.5)	0.26	>60.9[a]	2.7[a] (-1.6-20.6)	<4.0[a]	>58.7[a]	0.002
PY-M-21	>63.8[a]	>68.3[a]	>64.9[a]	>65.8[a]	~	>63.8[a]	>17.1[a]	>64.9[a]	>65.8[a]	1.3	~	2.8[bs] (2.6-2.9)	6.6[eb] (6.2-7.0)	6.0[eb] (5.6-6.5)	0.09 (n=3)	3.4[a] (0.6-16.0)	~	5.4[a] (5.3-5.7)	<4.1[a]	0.6 (n=3)
PY-M-23	>70.9[a]	>65.9[a]	61.4[a]	>65.4[a]	~	>70.9[a]	61.1[a]	>31.9[a]	>65.4[a]	7.68	0.3[bs] (0.1-0.4)	2.9[bs] (2.6-3.2)	<0.2[eb]	<4.1[eb]	0.0002	3.1[a] (-0.1-15.2)	3.2[a] (0.4-3.9)	~	6.2[a] (2.6-9.0)	0.15 (n=3)
Quebec																				
Q-M-11	73[eb] (65.2-82.3)	23[eb] (9.8-45.7)	25.6[eb]	22.6[eb] (14.6-34.5)	~	43.5[a] (28.4-54.6)	~	16.7[a] (9.1-17.2)	11.2[a] (9.2-13.2)	~	4.7[eb] (4.2-5.3)	32.5[eb] (28.9-35)	28.5[eb] (26.0-30.5)	96.3[eb]	$1.9x10^{-3}$ (n=3)	<8.8[a]	<4.4[a]	5.6[a] (4.5-5.8)	43.7[a] (32.8-49)	0.31 (n=3)
Q-M-14	~	~	>100[a]	>100[a]	~	~	~	>100[a]	>100[a]	~	~	~	14.1[eb] (10.6-15.7)	8.8[eb] (5.2-15.5)	~	~	~	3.2[a] (0-12.4)	22.2[a] (0-58.2)	~
Q-M-16	~	3.3[a] (2.1-5.3)	>100[a]	>100[a]	~	~	2.9[a] (2.3-3.5)	>100[a]	>100[a]	$1x10^{-6}$ (n=3)	~	<0.04[a]	<0.04[a]	<0.04[a]	0.04 (n=3)	~	<0.24[a]	<0.24[a]	5.0[a] (0.03-7.16)	$6x10^{-7}$ (n=3)

TABLE B.1: RESULTS OF SUBLETHAL MARINE TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Menidia beryllina* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Menidia beryllina* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	Echinoderm Fertilization IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Champia parvula* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Q-M-29	7.4[e] (6.3-9.6)	19.1[e] (17.5-21.4)	>100[e]	>100[e]	-	7.5[e] (1.9-11.1)	22.4[ne]	>100[e]	>100[e]	-	4.8[ne] (1.6-5.9)	12.1[ne] (10-13.9)	1.1[ne] (0.6-1.6)	6.6[ne] (4.4-8.9)	-	0.2[ne]	0.2[e] (0-1.2)	0.8[e] (0-3.2)	17.4[e] (0-20.5)	-
Q-M-48	29.1[ne] (25.3-33.8)	18.4[ne] (16.1-21)	30.6[ne]	>100[ne]	-	12.9[e] (9-18.1)	-	-	-	-	1.3[ne] (1.2-1.6)	21.1[ne] (20.4-22)	0.83[ne] (0.81-0.86)	53.6[ne] (50-55.9)	$9.7x10^{-5}$	10.8[e] (0-13.5)	5.6[e] (0-10.8)	8.3[e] (5.8-12)	1.5[e] (1.3-1.8)	0.05
Atlantic																				
A-M-02	>100[e]	>100[e]	>100[e]	>100[e]	-	>100[e]	>100[e]	>100[e]	>100[e]	100	7.5[ne] (6.5-8.8)	6.2[ne] (3.8-7.7)	1.9[ne] (0.9-2.6)	1.1[ne] (0.8-5.6)	0.02	1.1[e] (0.1-2.6)	2.2[e] (1.1-3.2)	5.4[e] (0-12.1)	14.7[e] (0-15.9)	0.009
A-M-03	-	-	-	-	-	>100[e]	52[e] (8.2-70)	>100[e]	>100[e]	14.68	2.3[ne] (2.1-2.5)	0.9[ne] (0.8-1.0)	2.8[ne] (2.6-2.9)	3.9[ne] (2.9-4.4)	0.07	6.6[e] (0.03-12)	<4.4[e]	25[e] (11-44)	25[e] (11-37)	0.09
A-M-04	-	-	-	-	-	6.7[e] (4.6-7.9)	5.7[e] (4.2-9.2)	7[e] (5.2-8.8)	7.2[e] (6-8.7)	3.78	2.9[ne] (2.5-3.7)	1.1[ne] (0.9-1.3)	3.7[ne] (3.4-4.0)	3.7[ne] (2.5-4.9)	0.12	<4.4[e]	<4.4[e]	<4.4[e]	<4.4[e]	4.4
A-M-05	-	-	-	-	-	6.8[e] (5.9-7.4)	3[e] (2-4.1)	6.3[e] (5.4-6.9)	10.5[e] (6.6-18.7)	0.37	15.7[ne] (15.5-15.8)	15.8[ne] (15.2-16.1)	15.8[ne] (15.3-16.2)	15.1[ne] (14.1-15.9)	13.9	<4.4[e]	<4.4[e]	7.1[e]	<4.4[e]	1.37
A-M-06	>100[e]	>100[e]	>100[e]	>100[e]	-	>100[e]	>100[e]	>100[e]	>100[e]	100	46.6[e]	53.5[e]	56.3[e]	30.1[e]	9.84	10.9[e]	4.6[e]	12.2[e]	14.5[e]	0.62
A-M-07	16.2[e] (14.7-16.7)	17.8[e] (16.4-19.1)	>11.0[e]	25.4[e] (17-41)	-	>11.0[e]	21.9[e]	>11.0[e]	>33.0[e]	0.93	5.7[ne] (4.4-11.4)	12.2[ne] (0-25)	22.6[ne] (20.9-25)	0.04[ne] (0.02-0.83)	$5x10^{-7}$	4.7[e] (0-9.1)	4.5[e] (0.4-5.7)	5.3[e] (0-14.8)	2.2[e] (0-6.2)	0.47
A-M-09	-	-	-	-	-	5.4[e]	16[e]	12.9[e]	6.83[e]	0.59	16.4[e]	21.7[e]	10.3[e]	7.98[e]	1.16	1.14[e]	10.33[e]	7.72[e]	1.2[e]	0.008
A-M-10	-	-	-	-	-	8.1[e]	14.7[e]	28.6[e]	21.5[e]	0.88	1.9[e]	0.2[e]	7.1[e]	2.1[e]	0.0005	1.3[e]	1.9[e]	6.4[e]	2.1[e]	0.06
A-M-11	-	-	-	-	-	88.3[e]	>100[e]	>100[e]	>100[e]	69.4	2.8[e]	4.2[e]	1.2[e]	8.21[e]	0.044	6.71[e]	19.23[e]	4.59[e]	8.57[e]	0.326
A-M-13	-	-	-	-	-	8.4[e]	11.3[e]	4.96[e]	65.5[e]	0.03	10.9[e]	9.57[e]	7.56[e]	8.87[e]	4.03	5.48[e]	6.5[e]	1.26[e]	1.33[e]	0.02
A-M-14	-	-	-	-	-	89[e]	74.8[e]	85[e]	>100[e]	45.5	2.0[e]	1.1[e]	0.13[e]	4.71[e]	0.0003	2.26[e]	1.61[e]	6.3[e]	6.43[e]	0.05
A-M-16	6.3[e]	34.6[e]	81.8[e]	20.2[e]	-	10.5[e]	>50[e]	>100[e]	>50[e]	0.24	1.8[e]	1.6[e]	3.9[e]	<3.4[e]	0.23	0.4[e]	<0.24[e]	0.32[e]	1.4[e]	0.007
A-M-17	4.8[e] (4.4-5)	>3.7[e]	6.3[e] (5.6-9.4)	>11.0[e]	-	>3.3[e]	>3.7[e]	6.3[e] (5.2-7.3)	>11.0[e]	0.24 (n=3)	0.4[ne] (0.3-0.5)	0.2[ne] (0.1-0.5)	0.7[ne] (0.6-0.8)	0.14[ne] (0.08-0.21)	$1.2x10^{-3}$ (n=3)	<0.2[e]	0.4[e] (0-0.8)	<0.2[e]	11.7[e] (3.9-17.9)	$8.8x10^{-3}$ (n=3)

TABLE B.2: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Ceriodaphnia dubia* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Ceriodaphnia dubia* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Selenastrum capricornutum* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Lemna minor* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Pacific & Yukon																				
PY-F-02	>100*	>100*	>100*	>100*	-	17.7* (13.8-19.1)	21.6* (18.3-26.7)	20.1* (16.9-25.0)	42.2* (38.3-47.7)	2.96	8.4* (6.0-12.3)	77.3* (72.5-79.4)	>100*	7.7* (4.4-10.3)	0.016	-	-	-	-	-
PY-F-03	>100*	>100*	>100*	>100*	-	96.7*	71.6* (24.8-86.9)	38.6* (18.6-50.2)	>100*	6.73	18.4* (-3.4-26.0)	51.3* (16.9-62.8)	67.8* (61.7-72.3)	83* (66.2-93.6)	1.32	-	-	-	-	-
PY-F-04	>100*	>100*	>100*	>100*	-	34.5* (11.2-58.7)	68*	78.8*	19.8*	1.41	>100**	>100**	>100**	>100**	-	-	-	-	-	-
PY-F-05	20.7* (17.1-29.9)	6.5* (2.8-7.8)	9.5* (8.2-13.3)	37.6* (32.8-53.1)	-	<6.25*	<6.2*	<6.2*	<6.2*	6.06	15.6* (11.3-17.2)	3* (2.1-3.7)	3.2* (2.8-3.4)	<1.0*	0.008	-	-	-	-	-
PY-F-08	>100*	>100*	>100*	>100*	-	31*	16*	32*	22*	5.63	>90.9*	50*	55*	41*	9.17	-	-	-	-	-
PY-F-09	64* (60-72)	27* (15-50)	56.2*	32.2*	-	4.4* (5.1-8.0)	3.6* (0.01-4.8)	47.5* (18.4-47.7)	15.4* (11.1-17.3)	0.016	5.6* (5.5-5.8)	11.6* (7.6-104.4)	21.7* (20.5-22.6)	4.2* (2.6-5.2)	0.17	-	-	-	-	-
PY-F-10	>100*	>100*	>100*	>100*	-	8.8* (6.0-10.7)	>100*	41.1* (28.0-61.3)	4.7* (2.5-6.9)	0.011	-	-	-	-	-	>100*	>100*	>100*	87.5* 47.7-98.5)	67.6
PY-F-13	>100*	>100*	>100*	>100*	-	31.1* (24.9-38.8)	23.6* (10.2-51.4)	11.5* (7.3-17.2)	53.7* (32.1-66.7)	0.83	-	-	-	-	-	>100*	>100*	>100*	>100*	100
PY-F-14	>100*	>100*	>100*	>100*	-	26.2* (21.3-34.4)	11* (4.5-16.7)	18.3* (15.4-25.2)	20.6* (17.8-22.8)	2.55	10.2* (7.9-12.4)	79.2* (74.2-82.8)	67.4* (60.2-73.5)	7.8* (-1.0-28.8)	0.04	-	-	-	-	-
PY-F-15	>100*	>100*	>100*	>100*	-	5*	65*	29*	14*	0.05	>90.9*	>90.9*	38*	41*	4.49	-	-	-	-	-
PY-F-19	>100*	>100*	22.6* (10.9-54.2)	>100*	-	21.2* (10.5-29.4)	12.2* (3.9-29.5)	10.6* (5.8-14.5)	20.8* (18.1-26.0)	2.25	4.3* (2.2-6.4)	6* (5.5-6.8)	2.7* (0.9-4.6)	<1.0*	0.044	-	-	-	-	-
PY-F-20	>100*	>100*	>100*	>100*	-	8* (4.9-9.7)	10* (4-26)	13* (10-20)	21* (11-29)	1.31	18* (15-20)	49* (45-51)	58* (52-63)	48* (43-52)	2.27	-	-	-	-	-
PY-F-22	>100*	>100*	>100*	>100*	-	32.6* (26.5-36.8)	34.6* (29.3-38.6)	22.2* (19.6-28.7)	39.2* (35.8-43.9)	8.44	21.3* (17.4-23.8)	43.2* (34.1-48.6)	64.1* (29.1-86.9)	25.3* (22.4-27.4)	2.33	-	-	-	-	-

TABLE B.2: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Ceriodaphnia dubia* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Ceriodaphnia dubia* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Selenastrum capricornutum* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Lemna minor* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Prairie & Northern																				
PN-F-01	>100[ns]	>100[ns]	>100[ns]	86[ns]	-	66[s] (11-80)	71[s] (55-91)	>100[s]	61[s] (55-66)	22.7	15[s] (12-19)	14[s] (13-15)	12[s] (9-15)	43[s] (14-61)	0.79	-	-	-	-	-
PN-F-02	>100[ns]	>100[ns]	>100[ns]	>100[ns]	-	5[s] (4-9)	7[s] (4-15)	9[s] (6-11)	23[s] (17-28)	0.27	79[s] (67-93)	9[s] (8-9)	79[s] (70-91)	>100[s]	0.11	-	-	-	-	-
PN-F-03	-	-	-	58[ns] (42-72)	-	-	-	-	26[s] (21-31)	-	-	-	-	21[s] (18-24)	-	-	-	-	-	-
PN-F-04	3.08[ns] (2.41-3.85)	-	-	-	-	1.61[s] (1.6-1.62)	1.45[s] (1.45-1.46)	>100[s]	>100[s]	-	-	-	-	-	-	38[ns] 31.5-49.3)	14.4[ns]	>100[ns]	>100[ns]	-
PN-F-05	>100[ns]	>100[ns]	>100[ns]	>100[ns]	-	33.5[s] (26.7-38.5)	51.1[s] (10.2-57.2)	85.5[s]	37.3[s] (32.1-39.0)	5.06	-	-	-	-	-	>100[s]	>100[s]	>100[s]	>100[s]	100
PN-F-06	15.7[s] (7.5-26.8)	>100[s]	43.6[s] (22.8-58.3)	60.5[s] (55.9-62.5)	-	8.4[s] (6.5-8.7)	4.9[s] (2.6-29.0)	14.6[s] (13.1-15.5)	10.7[s] (9.2-12.2)	0.75	3.5[s] (1.4-5.6)	25[s] (24.0-26.0)	30[s] (27.0-33.0)	44[s] (19.0-59.0)	0.04	-	-	-	-	-
PN-F-07	>100[ns]	>100[ns]	>100[ns]	>100[ns]	-	55[s] (43-63)	59[s] (52-67)	43[s] (36-51)	76[s] (65-86)	16.2	22[s] (18-24)	18[s] (10-35)	82[s] (46-98)	>100[s]	0.37	-	-	-	-	-
PN-F-08	>100[ns]	>100[ns]	>100[ns]	>100[ns]	-	67[s] (52.1-77.0)	54.8[s] (48.4-60.1)	54.3[s] (18.4-62.5)	40.7[s] (26.5-56.5)	17.7	79.9[s] (64.9-90.9)	68.2[s] (61.9-73.2)	94.4[s]	13.1[s] (7.9-16.5)	0.37	-	-	-	-	-
PN-F-09	>100[ns]	>100[ns]	>100[ns]	>100[ns]	-	64.1[s] (56.1-71.2)	47.8[s] (42.6-56.8)	22.4[s] (19.9-27.4)	56.2[s] (44.7-63.7)	3.55	-	-	-	-	-	>100[s]	>100[s]	>100[s]	>100[s]	100
Ontario																				
0-F-01	>3.3[ns]	7.8[ns] (3.7-33.0)	11[ns] (3.7-33.0)	>100[ns]	-	1.6[s] (1.1-1.8)	0.9[s] (0.6-1.2)	0.05[s] (0.03-1.28)	49.6[s] (28.9-66.3)	$7.2x10^{-6}$ (n=3)	-	-	-	-	-	18.8[s] 13.4-22.1	78.8[s] 66.7-87.5	20.3[s] (6.6-31.8)	>100[s]	0.036 (n=3)
0-F-02	5.7[ns] (3.7-11.0)	27.4[ns] (20.6-37.0)	19.1[ns] (11.0-33.0)	17.9[ns]	-	>3.7[s]	1.5[s] (1.0-2.6)	<0.14[s]	1.9[s] (1.2-2.0)	0.0005	-	-	-	-	-	48.5[s] 39.1-51.1)	96.4[ns]	>50.0[s]	59.9[s] 52.9-64.0)	11.14
0-F-03	>100[ns]	>100[ns]	>100[ns]	>100[ns]	-	34[s] (17-41)	22[s] (16-26)	22[s] (18-28)	5.8[s] (4.8-7.3)	0.29	-	-	-	-	-	>100[s]	83[s] (63-94)	>100[s]	>100[s]	57.88

TABLE B.2: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Ceriodaphnia dubia* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Ceriodaphnia dubia* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Selenastrum capricornutum* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Lemna minor* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
0-F-04	>100[lo]	>100[lo]	>100[lo]	>100[lo]	-	5.3[e] (3.7-7.2)	2.1[e] (1.4-2.9)	3.2[e] (1.3-3.7)	0.85[e] (0.76-1.1)	0.04	-	-	-	-	-	>100[e]	>100[e]	>100[e]	>100[e]	100
0-F-05	31.4[lo]	>3[lo]	34.3[lo]	75.1[lo]	-	1.7[p]	2.1[p]	2.1[p]	8[e]	0.7 (n=3)	-	-	-	-	-	32.1[p]	>100[p]	57.1[p]	>100[e]	0.28 (n=3)
0-F-06	100[hlo]	>100[lo]	>100[lo]	>100[lo]	-	26.6[p] (10.2-58.0)	10[p] (2.5-17.03)	59[p] (54.8-63.6)	26[p] (21-29)	0.51	19.1[p] (16.6-20.7)	18.9[p] (18.719.3)	19.8[p] (14.6-25.6)	30[p] (22-39)	6.52	-	-	-	-	-
0-F-07	>100[p]	>100[p]	>100[p]	>100[p]	-	<6.0[p]	12.5[p] (1.9-16.0)	74.7[e]	4.6[p] (3.9-6.1)	0.015	75.4[p] (72.5-77.6)	4.4[p] (2.5-6.9)	15.9[p] (10.7-23.7)	15.5[p] (7.0-35.9)	0.03	-	-	-	-	-
0-F-08	>100[lo]	65.0[lo]	>100[lo]	50[lo]	-	2.3[p] (1.1-4.0)	22.7[p] (11.1-26.6)	18.1[p] (14.7-23.8)	8.7[p] (4.9-10.6)	0.037	48.5[p] (39.8-54.7)	19.1[p] (18.4-19.6)	19.7[p] (16.1-22.6)	0.5[p] (0.24-0.91)	0.0002	-	-	-	-	-
0-F-09	4.8[lo]	7.1[lo]	0.6[lo]	10.9[lo]	-	0.39[p]	<0.39[p]	0.04[p] (0.02-0.08)	0.11[p] (0.11-0.12)	0.0004	0.8[p] (0.3-1.1)	6[p] (3.8-7.7)	0.2[p] (0.1-0.3)	0.26[p] (0.2-0.3)	0.0002	-	-	-	-	-
0-F-10	>100[lo]	>100[lo]	70.7[lo]	>100[lo]	-	>100[p]	17.6[p]	23.2[e]	23[e]	-	-	-	-	-	-	>100[p]	>100[p]	-	-	-
0-F-11	>100[lo]	>100[lo]	>100[lo]	>100[lo]	-	>100[e]	28[e] (42-55)	19[e] (24-38)	33[e] (40-61)	0.79	-	-	-	-	-	>100[e]	>100[e]	>100[e]	>100[e]	100
0-F-12	70.7[lo] (50-100)	66.0[lo] (57.8-75.2)	66.0[lo] (57.8-75.2)	66.0[lo] (62.7-73.8)	-	9.5[p] (6.2-15.3)	19.5[p] (6.0-25.9)	30.3[p] (28.9-35.1)	17.9[p] (13.9-20.2)	1.37	-	-	-	-	-	>100[p]	>100[p]	>100[p]	>100[p]	100
0-F-13	-	-	-	-	-	>100[e]	>100[e]	>100[e]	>100[e]	100	>100[e]	>100[e]	>100[e]	>100[e]	100	-	-	-	-	-
0-F-14	ND	>100[lo]	>100[lo]	>100[lo]	-	35[e] (30-38)	5.9[e] (4.0-15)	23[e] (2.7-62)	24[e] (16-33)	0.28	-	-	-	-	-	-	78[e] (52-95)	>100[e]	>100[e]	27.6 (n=3)
0-F-15	>100[e]	>100[e]	>100[e]	>100[e]	-	>100[e]	>100[e]	>100[e]	>100[e]	100	26.3[e] (20.4-32.3)	9[e] (1.7-14.1)	50.6[e] (31.3-71.3)	>100[e]	0.13	-	-	-	-	-
0-F-16	>100[lo]	>100[lo]	>100[lo]	>100[lo]	-	>100[e]	23.4[e] (9.7-66.1)	>100[e]	<6.0[e]	0.02	-	-	-	-	-	>100[e]	>100[e]	>100[e]	>100[e]	100
0-F-17	>100[lo]	>100[lo]	>100[lo]	-	-	17.6[e]	19.1[e]	12.1[e]	-	2.08 (n=3)	-	-	-	-	-	>100[e]	>100[e]	>100[e]	-	100 (n=3)
0-F-18	5.2[lo] (1.2-11.0)	35.4[lo] (25.0-50.0)	51.1[lo]	>100[lo]	-	2.6[lo]	23.3[lo]	9.2[p] (3.4-16.5)	34.5[p] (26.0-36.2)	0.02	-	-	-	-	-	>100[p]	>100[p]	>100[p]	>100[p]	100

TABLE B.2: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Ceriodaphnia dubia* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Ceriodaphnia dubia* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Selenastrum capricornutum* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Lemna minor* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Q-F-19	>100[a]	>100[a]	>100[a]	>100[a]	-	<6.0[a]	11.6[a] (5.5-17.4)	46.3[a] (41.4-52.8)	63.2[a] (54.6-63.8)	0.05	>100[a]	>100[a]	>100[a]	>100[a]	100	-	-	-	-	-
Q-F-20	41[bc] (31-54)	29[bc] (24-35)	53[bc] (48-58)	>100[bc]	-	4.3[a] (2.4-5.9)	2.9[a] (2.4-3.4)	3.4[a] (0.3-4.9)	45[a] (0.9-52)	1.2 (n=3)	-	-	-	-	-	>100[a]	39[a] (37-40)	44[a] (39-49)	>100[a]	0.76 (n=3)
Q-F-21	23[bc] (13.0-50.0)	16.8[bc] (13.0-25.0)	51.1[bc] (33.0-100)	19.1[bc] (11.0-33.0)	-	1.5[a] (1.1-2.1)	1.3[a] (1.2-1.4)	0.9[a] (0.7-1.0)	4.8[a] (3.5-5.7)	0.035	21.7[a] (0.9-32.4)	23.2[a] (21.2-24.2)	3.1[a] (0.8-6.0)	45.5[a] (18.9-70.6)	0.03	-	-	-	-	-
Q-F-22	63[bc] (47-85)	55[bc] (30-100)	>100[bc]	64[bc] (38-143)	-	0.6[a] (0.6-0.7)	0.3[a] (0.2-0.8)	3.3[a] (0.8-4.8)	0.09[a] (0.07-2.2)	0.0006	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
Q-F-23	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	32.3[a]	5.6[a]	16.3[a]	28.7[a]	0.23	15.3[ca]	19.7[ca]	33.9[ca]	>100[ca]	-	-	-	-	-	-
Q-F-24	-	-	-	-	-	0.98[a]	1.5[a]	0.9[a]	27[a]	0.25 (n=3)	4.1[ca]	6.3[ca]	5.7[ca]	83[ca]	-	-	-	-	-	-
Q-F-25	-	-	-	-	-	34[a] (23-43)	4.3[a] (4-5)	47.8[a] (32-58)	24.2[a] (20-30)	0.06	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
Q-F-26	>100[a]	>100[a]	>100[a]	>100[a]	-	7.1[a] (3.0-14.2)	24.8[a] (11.6-27.7)	47.4[a] (36.5-54.6)	42.1[a] (11.4-57.1)	0.23	24.2[a] (21.6-26.6)	9.5[a] (6.5-14.9)	21.4[a] (9.2-32.7)	9.1[a] (3.1-13.5)	0.89	-	-	-	-	-
Quebec																				
Q-F-03	-	70.7[bc] (50.0-100)	>100[bc]	65.7[bc] (12.5-100)	-	-	15.6[a] (13.2-18.0)	14.9[a] (<6.3-31.4)	6.0[a] (<6.3-20.4)	0.12 (n=3)	-	9.4[a] (8.2-10.6)	36.2[a] (33.1-39.3)	26.0[a] (9.1-42.9)	0.057 (n=3)	-	-	-	-	-
Q-F-04	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[a]	8.7[a] (7.7-9.5)	48.6[a] (31.4-65.8)	59.6[a] (46.6-72.6)	0.135	>100[a]	47.6[a] (41.3-53.8)	32.3[a] (28.2-36.4)	91.3[a] (86.0-96.6)	3.38	-	-	-	-	-
Q-F-05	8.3[bc] (6.2-11.1)	52.5[bc] (36.9-83.0)	>100[bc]	>100[bc]	-	4.4[a] (3.9-5.3)	6.5[a] (4.7-8.3)	25.5[a] (19.6-31.4)	0.5[a] (<0-4.8)	0.00069	4.3[a] (<0-9.4)	3.9[a] (<0-9.7)	10.2[a] (9.8-10.5)	3.2[a] (2.9-3.1)	0.31	-	-	-	-	-
Q-F-06	10.9[bc] (4.9-24.5)	>100[bc]	35.2[bc] (31.1-39.4)	>100[bc]	-	0.3[a] (0.25-0.29)	>100[a]	1.0[a] (0.2-2.1)	26.2[a] (20.0-32.5)	$2.2x10^{-11}$ (n=3)	4.8[a] (4.2-5.4)	>100[a]	0.81[a] (0.7-0.9)	97.5[a]	$1x10^{-6}$ (n=3)	-	-	-	-	-
Q-F-07	13.5[bc] (8.2-21.2)	27.3[bc] (3.1-50)	16.2[bc] (10.5-28.7)	23.9[bc] (16.8-34.2)	-	0.65[a] (0.43-0.87)	0.8[a] (0.6-1.0)	0.5[a] (<0-5.9)	0.2[a] (<0-4.2)	0.0179	1.1[a] (<0-5.6)	0.9[a] (<0-4.2)	0.3[a] (<0-4.5)	0.4[a] (<0-5.6)	0.0204	-	-	-	-	-
Q-F-08	57.4[bc] (45.7-72.1)	68.0[bc] (62.7-73.8)	51.2[bc] (44.5-59.0)	>100[bc]	-	2.2[a] (1.2-4.3)	0.6[a] (0.5-0.7)	0.63[a] (0.18-0.94)	91.9[a]	0.002 (n=3)	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100 (n=3)

TABLE B.2: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Ceriodaphnia dubia* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Ceriodaphnia dubia* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Selenastrum capricornutum* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Lemna minor* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Q-F-10	41.2[ns] (29.8-60.7)	46.6[ns] (35.9-65.0)	>100[ns]	>100[ns]	-	10.6[s] (9.0-12.9)	19.4[s] (15.1-25.2)	20.8[s] (9.55-26.5)	5.86[s] (2.65-13.2)	0.726 (n=3)	-	-	-	-	-	>100[s]	>100[s]	>100[s]	>100[s]	100 (n=3)
Q-F-12	45.8[ns] (32-100)	17.9[ns] (10-32)	>100[ns]	>100[ns]	-	1.1[s] (0.4-5.2)	0.32[s] (0.19-0.58)	18.4[s] (10.1-23.3)	>100[s]	-	6.27[s] (1.34-12.3)	6.62[s] (0.97-27.3)	1.07[s] (0.82-1.48)	1.37[s] (1.05-1.87)	-	-	-	-	-	-
Q-F-13	5.3[ns] (2.8-9.3)	-	>100[ns]	100[ns] (25->100)	-	0.34[s] (<0-12.7)	64.5[s] (32.7-98.3)	49.2[s] (21.2-77.3)	10.2[s] (3.4-17.0)	$7.6x10^{-3}$ (n=3)	4[s] (0.8-7.2)	9.2[s] (1.5-16.8)	8.9[s] (7.4-10.4)	24.5[s] (20.3-28.7)	0.1 (n=3)	-	-	-	-	-
Q-F-15	-	-	>100[ns]	>100[ns]	-	43.1[s] (16.6-69.5)	50.3[s] (48.8-51.9)	>100[s]	17.8[s] (2.5-33.0)	0.98	23.5[s] (11.3-35.7)	22.4[s] (2.7-42.1)	22.7[s] (21.3-24.2)	17.7[s] (11.6-23.9)	10.7	-	-	-	-	-
Q-F-17	1.9[ns] (1.2-3.2)	32.9[ns] (25-50)	43.2[ns] (32.457.7)	>100[ns]	-	0.19[s] (<0-5.6)	0.66[s] (<0-14.5)	24.6[s] (9.9-39.4)	12[s] (8.6-15.4)	$6.2x10^{-7}$ (n=3)	0.9[s] (<0-4.8)	11.1[s] (9.4-12.9)	10.5[s] (4.6-16.4)	15.2[s] (8.3-22.0)	2.27 (n=3)	-	-	-	-	-
Q-F-18	13.9[ns] (9.3-21.3)	-	>100[ns]	>100[ns]	-	0.18[s] (<0-6.0)	46.8[s] (27.2-66.4)	73.8[s] (73.6-74.0)	23.9[s] (15.9-31.9)	0.37 (n=3)	5[s] (1.5-8.6)	16.3[s] (12.3-20.2)	>100[s]	12.8[s] (0.0-25.5)	$2.24x10^{-3}$ (n=3)	-	-	-	-	-
Q-F-19	45.8[ns] (25-100)	54.6[ns] (25-100)	>100[ns]	>100[ns]	-	6.7[s] (2.9-9.3)	15.6[s] (5.2-26.0)	>100[s]	>100[s]	-	19.3[s] (12.2-26.4)	6.8[s] (0-30.3)	32.2[s] (33.0-33.3)	20.9[s] (12.5-29.2)	-	-	-	-	-	-
Q-F-20	-	-	-	-	-	<1.0[s]	0.3[s] (0.2-0.3)	0.3[s] (0.2-0.4)	-	$1.3x10^{-3}$ (n=3)	16.2[s] (2.9-51.3)	-	5.0[s] (2.7-8.0)	-	-	-	-	-	-	-
Q-F-21	7.5[ns] (4.1-13.4)	5.1[ns] (2.8-10.1)	>100[ns]	>100[ns]	-	0.3[s] (0.29-0.33)	0.2[s] (0.2-0.22)	2.8[s] (0.1-38.4)	52.5[s] (45-60)	-	0.6[s] (0.5-1.0)	0.2[s] (0.2-0.4)	1.0[s] (0.7-2.0)	>100[s]	-	-	-	-	-	-
Q-F-22	35.4[ns] (25-50)	17.7[ns] (12.5-25)	17.7[ns] (12.5-25)	>100[ns]	-	8.9[s] (5.7-11.3)	9.1[s] (0.4-17.7)	0.9[s] (<0-3.7)	55.5[s] (27.4-83.6)	$6x10^{-5}$ (n=3)	10.4[s] (<0-24.0)	0.6[s] (<0-4.3)	8.6[s] (1.7-15.4)	13.9[s] (6.8-21.1)	$6x10^{-6}$ (n=3)	-	-	-	-	-
Q-F-23	>100[ns]	>100[ns] (50-100)	>100[ns]	>100[ns]	-	7.5[s] (5.6-8.8)	19.1[s] (11.7-26.5)	22.2[s] (0-66.9)	>100[s]	0.11 (n=3)	11.6[s] (9.6-13.6)	5.1[s] (1.1-9.1)	10.5[s] (0-21.9)	14.6[s] (10.4-18.7)	0.2 (n=3)	-	-	-	-	-
Q-F-24	4.7[ns] (3.6-6.2)	8.8[ns] (6.3-12.5)	>100[ns]	>100[ns]	-	0.5[s] (0.5-0.6)	0.1[s] (<0-9.6)	31[s] (24.0-37.9)	16.5[s] (15.4-17.7)	-	4.1[s] (<0-9.8)	4.6[s] (<0-10.0)	4.5[s] (<0-10.2)	4.3[s] (2.6-6.0)	-	-	-	-	-	-
Q-F-25	57.2[ns] (44.5-77.5)	>100[ns]	82.2[ns] (49.0->100)	>100[ns]	-	14.8[s] (5.9-20.9)	34.1[s] (22.9-45.3)	12.2[s] (<0-24.7)	9.5[s] (8.7-10.2)	0.794	38.3[s] (30.9-45.8)	7[s] (3.6-10.5)	12.2[s] (4.4-19.9)	21.2[s] (18.4-24.1)	0.323	-	-	-	-	-
Q-F-26	>100[ns]	>100[ns]	>100[ns]	>100[ns]	-	>100[s]	>100[s]	100[s]	>100[s]	100	12.3[s] (2.3-22.3)	>100[s]	>100[s]	73.4[s] (63.7-83.2)	0.246	-	-	-	-	-

TABLE B.2: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Ceriodaphnia dubia* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Ceriodaphnia dubia* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Selenastrum capricornutum* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Lemna minor* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Q-F-27	>100[NC]	>100[NC]	70.7[NC]	>100[NC]	-	>100[P]	72.5[P]	23.2[P]	23.0[P]	-	-	-	-	-	-	>100[P]	>100[P]	>100[P]	>100[P]	-
Q-F-28	70.7[NC] (50-100)	2.2[NC] (1.7-2.9)	17.3[NC] (10-30)	5.12[NC] (4.45-5.90)	-	5.8[P] (4.2-7.7)	0.5[P] (0.3-0.9)	3.1[P] (0.1-6.9)	2.05[P] (0.73-3.49)	0.008	-	-	-	-	-	>100[P]	78.8[P] (48.0-89.3)	>100[P]	93[P]	50.61
Q-F-31	40[NC] (30.8-53.9)	35.4[NC] (25.0-50.0)	>100[NC]	32.9[NC] (1.6-50.0)	-	2.0[P] (1.6-2.4)	0.5[P] (0.5-0.6)	30.4[P] (25.7-35.0)	0.8[P] (<0-10.4)	$1.2x10^{-4}$	11.5[P] (9.7-14.3)	6.8[P] (<0-17.4)	36.5[P] (31.7-41.4)	3.0[P] (1.0-5.1)	0.0348	-	-	-	-	-
Q-F-32	11.5[NC] (8.6-15.4)	7.6[NC] (4.7-14.6)	14.5[NC] (8.9-25.1)	>100[NC]	-	0.6[P] (0.5-0.7)	0.61[P] (0.56-0.68)	0.11[P] (<0-18.7)	>100[P]	$9x10^{-5}$ (n=3)	7.2[P] (6.97.5)	8.1[P] (0.1-16.1)	10.2[P] (<0-25.8)	34.8[P] (23.4-46.3)	1.91 (n=3)	-	-	-	-	-
Q-F-34	13.6[NC] (6.3-25.0)	17.7[NC] (0.39-25.0)	32.9[NC] (0.39-50.0)	>100[NC]	-	0.6[P] (0.5-0.7)	0.21[P] (<0-9.3)	0.24[P] (<0-19.9)	>100[P]	0.003 (n=3)	1.9[P] (<0-4.5)	1.5[P] (<0-3.5)	5.3[P] (0.1-10.5)	12.6[P] (9.4-15.9)	0.009 (n=3)	-	-	-	-	-
Q-F-35	16.4[NC] (12.5-25.0)	11.4[NC] (6.3-25.0)	17.7[NC] (12.5-25.0)	>100[NC]	-	3.4[P] (3.0-3.8)	2.9[P] (2.1-3.5)	1.0[P] (<0-21.0)	57.3[P]	0.008 (n=3)	21.1[P] (17.9-24.8)	25.8[P] (21.7-30.0)	42.6[P] (34.0-51.1)	30.3[P] (25.3-35.2)	1.375 (n=3)	-	-	-	-	-
Q-F-36	>33.0[NC]	57.4[NC] (33.0-100)	57.4[NC] (33.0-100)	23.6[NC] (14.8-37.2)	-	0.2[P] (0.1-0.3)	0.6[P] (0.3-1.2)	2.5[P] (1.6-3.3)	1.6[P] (0.3-3.6)	0.002	2.5[P] (2.2-2.7)	4.9[P] (0.7-9.7)	2.8[P] (0.6-11.8)	1.0[P] (0.6-2.0)	0.07	-	-	-	-	-
Q-F-37	20.5[NC] (15.5-27.1)	100[NC]	46.8[NC] (11.0-100)	57.4[NC] (33.0-100)	-	1.2[P] (1.0-1.8)	12[P] (7.3-13.8)	12.4[P] (8.0-15.5)	18.9[P] (14.8-22.3)	0.009	2.1[P] (0.1-3.5)	8.9[P] (3.2-14.2)	5.4[P] (2.8-7.9)	0.8[P] (0.6-1.8)	0.0098	-	-	-	-	-
Q-F-39	2.4[NC] (1.5-3.9)	8.8[NC] (0.4-12.5)	79.6[NC] (6.3->100)	>100[NC]	-	0.05[P] (0.3-0.7)	0.05[P] (<0-3.9)	13.3[P] (12.0-14.7)	45.1[P] (22.5-67.6)	-	1.3[P] (<0-13.7)	0.4[P] (<0-5.7)	3.1[P] (2.6-3.6)	4.3[P] (0-15.1)	-	-	-	-	-	-
Q-F-42	27.3[NC]	>100[NC]	100[NC]	70.7[NC]	-	0.5[P]	0.6[P]	1.3[P]	0.7[P]	0.078	27.6[P]	3.39[P]	0.876[P]	3.22[P]	0.0019	-	-	-	-	-
Q-F-44	7.2[NC] (3.7-11.0)	4.6[NC] (3.0-6.0)	6.4[NC] (3.7-11.0)	9.6[P] (3.7-33.0)	-	0.3[P] (0.2-0.5)	<0.75[P]	0.08[P] (0.04-0.12)	0.2[P] (<0.2-0.3)	0.008	-	-	-	-	-	>100[P]	45.6[P]	51.3[P]	78.8[P]	9.278
Q-F-46	57.4[NC] (33.0-100)	70.7[NC] (50-100)	51.1[NC] (33-100)	>100[NC]	-	2.2[P] (1.7-2.4)	2.3[NC]	1.8[P] (0.9-2.3)	16.7[P] (11.2-18.3)	0.7 (n=3)	-	-	-	-	-	>100[P]	>100[P]	>100[P]	>100[P]	100 (n=3)
Q-F-48	74.6[NC] (56-100)	21.4[NC] (12.6-36.7)	13.4[NC] (7.3-25.7)	>100[NC]	-	0.33[P] (0.28-0.36)	0.33[P] (0.31-0.36)	0.19[P] (0.14-0.31)	20.3[P] (17.6-23.2)	0.019 (n=3)	>100[P]	5.4[P] (3.2-9.9)	1.36[P] (1.0-1.63)	4.2[P] (0.8-5.5)	$9.3x10^{-6}$ (n=3)	-	-	-	-	-
Q-F-47	>100[NC]	>100[NC]	57.4[NC] (33-100)	42.5[NC] (11-100)	-	1.8[P] (0.8-2.6)	2.2[P] (1.0-4.7)	0.4[P] (0.2-0.8)	4.0[P] (2.7-6.1)	0.008	-	-	-	-	-	67.3[P] (57-72.3)	>100[P]	89.2[NC]	>100[P]	32.27

TABLE B.2: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	*Ceriodaphnia dubia* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Ceriodaphnia dubia* Reproduction IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Selenastrum capricornutum* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Lemna minor* Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Q-F-49	>100[bp]	>100[bp]	3.5[bs] (2.5-4.9)	>100[bs]	-	4.4[p] (<0-13.3)	0.57[p] (<0-18.7)	>3.1[s]	78.7[s] (65.4-91.9)	-	79.1[p] (73.1-85.1)	27.1[p] (20.3-34.0)	49.6[s] (45.3-53.8)	23.3[s] (20.8-25.8)	-	-	-	-	-	-
Q-F-50A	-	70.7[bp] (50-100)	>100[bp]	65.7[bs] (12.5-100)	-	-	15.6[p] (13.2-18.0)	14.9[p] (<0-31.4)	6.0[s] (<0-20.4)	-	-	9.4[p] (8.2-10.6)	36.2[p] (33.1-39.3)	26.0[s] (9.1-42.9)	-	-	-	-	-	-
Q-F-50B	>100[bp]	22.9[bp] (12.5-50.0)	22.7[bp] (16.1-33.7)	7.8[bp] (3.13-12.5)	-	16.4[p] (14.5-17.6)	1.6[p] (1.2-2.1)	1.8[p] (0.9-2.6)	2.8[p] (<0-14.0)	0.01	3.2[p] (<0-10.1)	2.0[p] (<0-9.9)	6.1[p] (4.2-8.0)	3.9[p] (<0-7.9)	0.293	-	-	-	-	-
Q-F-52	>100[bs]	>100[bs]	>100[bs]	76.2[bs] (62.1-93.6)	-	30.2[s] (26.5-32.5)	>100[s]	18.8[s] (10.1-26.9)	18.9[s] (5.8-26.6)	0.469	-	-	-	-	-	>100[s]	>100[s]	>100[s]	>100[s]	100
Q-F-53	58.6[bs] (25-100)	>100[bs]	44.4[bs] (33.8-58.3)	57.3[bs] (44.1-74.4)	-	14.5[s] (8.5-16.4)	22.3[s] (12.2-34.6)	7.1[s] (3.0-9.0)	3.63[s] (2.1-5.3)	0.131	-	-	-	-	-	>100[s]	>100[s]	>100[s]	>100[s]	100
Q-F-55	3.8[bs] (3.2-4.6)	4.0[bs] (3.1-5.0)	>100[bs]	>100[bs]	-	<0.39[p]	0.08[p] (0.05-0.2)	71.6[s] (64-77)	16.9[s] (10-22)	-	-	-	-	-	-	>100[p]	>100[p]	>100[s]	100[s]	-
Q-F-58	>100[bs]	35.4[bs] (25.9-48.2)	>100[bs]	62.2[bs] (12.5-100)	-	52.2[s] (46.9-58.3)	6.4[s] (5.0-7.8)	47.3[s] (10.7-83.9)	23.2[s] (7.4-24.9)	0.136	25.8[s] (9.5-42.1)	2.8[s] (0.1-5.5)	1.0[s] (<0-3.4)	13.1[s] (<0-27.1)	0.002	-	-	-	-	-
Q-F-63	>100[bs]	>100[bs]	>100[bs]	>100[bs]	-	-	-	-	-	-	>100[s]	>100[s]	>100[s]	>100[s]	100	-	-	-	-	-
Q-F-64	>100[bs]	15.8[bs] (10.0-32.0)	>100[bs]	75.1[bs] (36.4->100)	-	0.31[s] (0.28-0.34)	6.7[s] (2.8-9.7)	6.83[s] (0.59-27.1)	8.9[s] (7.6-10.2)	0.0006	2.45[s] (1.09-3.63)	0.22[s] (0.17-0.36)	1.12[s] (0.84-1.51)	1.01[s] (0.85-1.25)	0.0039	-	-	-	-	-
Atlantic																				
A-F-01	-	-	-	-	-	<1.5[p]	1.8[p] (1.2-2.2)	0.6[p] (0.1-1.9)	20.3[s] (12.1-26.0)	$8.4x10^{-3}$ (n=3)	-	-	-	-	-	>100[p]	>100[p]	>100[p]	>100[s]	100 (n=3)
A-F-08	-	-	-	-	-	<6.0[p]	2.6[p] (2.4-3.5)	10.1[s] (8.6-12.8)	17.6[s] (14.0-18.6)	0.09	21.0[p] (14.3-46.2)	<1.0[p]	18.3[s] (11.4-26.0)	24.5[s] (23.9-25.1)	0.003	-	-	-	-	-
A-F-12	-	-	-	-	-	1.6[s]	2.8[s]	27.7[s]	0.4[s]	0.0002	-	-	-	-	-	72[s]	61.7[s]	75.6[s]	>100[s]	25.8
A-F-15	-	-	-	-	-	9.2[s] (8.2-10.4)	10.9[s] (8.2-13.8)	17.8[s] (13-19)	18.1[s] (15-19)	2.11	-	-	-	-	-	>100[s]	>100[s]	>100[s]	>100[s]	100

[a] IC50
[b] LC50
[c] EC50
[d] Estimated value
[p] Primary treatment
[s] Secondary treatment

TABLE B.3: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	Fathead Minnow Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	Fathead Minnow Growth IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)	*Oncorhynchus mykiss* Survival IC25 (95% CI) Quarters 1st	2nd	3rd	4th	PMTC (%)
Pacific & Yukon															
PY-F-02	-	-	-	-	-	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
PY-F-03	-	-	-	-	-	-	-	-	-	-	<6.25[a]	>100[a]	>100[a]	>100[a]	0.03
PY-F-04	-	-	-	-	-	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
PY-F-05	-	-	-	-	-	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
PY-F-06	-	-	-	-	-	-	-	-	-	-	14[a]	48[a]	42[a]	14[a]	0.67
PY-F-09	>100[a]	58.3[a]	>100[a]	>100[a]	-	79[a]	>50[a]	>100[a]	>100[a]	13.7	-	-	-	-	-
PY-F-10	-	-	-	-	-	-	-	-	-	-	>100[a]	50[a] (42.2-55.4)	20.3[a] (19.1-21.6)	71.7[a] (66.3-75.6)	1.3
PY-F-13	-	-	-	-	-	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
PY-F-14	-	-	-	-	-	-	-	-	-	-	61.2[a] (55.8-64.6)	59.1[a] (53.5-62.8)	56.2[a] (44.8-63.9)	62.9[a] (56.5-68.0)	39.8
PY-F-15	-	-	-	-	-	-	-	-	-	-	13.7[a] (12.6-14.7)	20[a]	27[a] (19.5-44.9)	12.4[a] (10.9-14.3)	2.55
PY-F-19	-	-	-	-	-	-	-	-	-	-	<6.25[a]	>100[a]	63.8[a]	>100[a]	0.04
PY-F-20	-	-	-	-	-	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
PY-F-22	-	-	-	-	-	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100
Prairie & Northern															
PN-F-01	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[a]	>100[a]	>100[a]	>100[a]	100	-	-	-	-	-
PN-F-02	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[a]	>100[a]	46[a] (35-50)	>100[a]	10.2	-	-	-	-	-
PN-F-03	-	-	-	85[bc] (72-99)	-	-	-	-	-[d]	-	-	-	-	-	-

TABLE B.3: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	Fathead Minnow										*Oncorhynchus mykiss*				
	Survival					Growth					Survival				
	IC25 (95% CI)				PMTC (%)	IC25 (95% CI)				PMTC (%)	IC25 (95% CI)				PMTC (%)
	Quarters					Quarters					Quarters				
	1st	2nd	3rd	4th		1st	2nd	3rd	4th		1st	2nd	3rd	4th	
PN-F-04	1.64[bc] (1.38-1.97)	-	-	-	-	-[e]	-	>100[e]	>100[e]	-	-	-	-	-	-
PN-F-05	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[e]	>100[e]	>100[e]	>100[e]	100	-	-	-	-	-
PN-F-06	>100[e]	>100[e]	>100[e]	>100[e]	-	>100[e]	>100[e]	>100[e]	>100[e]	100	-	-	-	-	-
PN-F-07	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[e]	>100[e]	>100[e]	>100[e]	100	-	-	-	-	-
PN-F-08	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[e]	>100[e]	95.2[e]	>100[e]	86.6	-	-	-	-	-
PN-F-09	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[e]	>100[e]	98[e] (88.3-99.4)	>100[e]	94.2	-	-	-	-	-
Ontario															
O-F-01	>3.3[c]	<3.7[eo]	<3.7[eo]	>100[e]	-	2.4[e] (1.8-2.6)	2.1[e] (1.1-2.4)	2.4[e] (1.9-2.5)	>100[e]	1.2 (n=3)	-	-	-	-	-
O-F-02	6.4[c] (5.7-7.2)	7.9[c] (7.7-8.0)	<3.7[eo]	6.4[c] (5.5-14.3)	-	6[c] (5.1-6.6)	5[c] (3.9-5.7)	2.7[c] (2.3-3.0)	5.6[c] (3.0-7.4)	0.65	-	-	-	-	-
O-F-03	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	83[e] (56-97)	57[e] (51-61)	>100[e]	89[e] (62-98)	21.8	-	-	-	-	-
O-F-04	60[bc] (59-85)	>100[bc]	>100[bc]	>100[bc]	-	21[e] (14-30)	68[e] (57-73)	34[e] (24-39)	42[e] (38-44)	2.76	-	-	-	-	-
O-F-05	>100[c]	1.8[c]	5.8[c]	25.7[e]	-	4.4[c]	>1.2[c]	2.9[c]	15.7[e]	9.6×10^{-3} (n=3)	-	-	-	-	-
O-F-06	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	14.9[c] (5.3-26.8)	22.8[c] (2.9-31.5)	35.3[c] (14.4-49.2)	34[c] (21-56)	2.89	-	-	-	-	-
O-F-07	5.2[c] (4.3-5.8)	5.1[eo] (3.2-6.3)	3.1[eo] (2.3-5.6)	2.6[c] (0-6.9)	-	20.9[c] (0.7-46.4)	6.6[eo] (1.5-12.2)	8.2[bc]	8.7[c] (3.9-13.0)	0.65	-	-	-	-	-
O-F-08	15[bc]	17.5[bc]	34.6[bc]	13.6[bc]	-	-[e]	6.9[c] (4.9-9.2)	10.6[c] (7.4-17.3)	10.5[c] (8.8-11.5)	1.173 (n=3)	-	-	-	-	-

TABLE B.3: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	Fathead Minnow										*Oncorhynchus mykiss*				
	Survival					Growth					Survival				
	IC25 (95% CI)					IC25 (95% CI)					IC25 (95% CI)				
	Quarters				PMTC (%)	Quarters				PMTC (%)	Quarters				PMTC (%)
	1st	2nd	3rd	4th		1st	2nd	3rd	4th		1st	2nd	3rd	4th	
O-F-09	7.5[bc]	4.2[bc]	2.9[bc]	12.0[bc]	-	1.6[b] (1.4-2.0)	1.6[b] (1.0-3.5)	-[b]	3.6[b] (2.8-4.8)	0.042 (n=3)	-	-	-	-	-
O-F-10	>100[bc]	16.7[bc]	41.5[bc]	>100[bc]	-	>100[b]	15.6[b]	-[b]	>100[b]	-	-	-	-	-	-
O-F-11	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[a]	>100[a]	>100[a]	>100[a]	100	-	-	-	-	-
O-F-12	30.5[bc] (25.9-35.9)	15.6[bc] (13.6-18.2)	31.6[bc] (28.5-35.4)	26.7[bc] (24.0-29.7)	-	15.6[b] (14.8-16.5)	11.5[b] (10.5-12.1)	18.2[b] (15.3-19.8)	15[b] (12.1-17.1)	5.34	-	-	-	-	-
O-F-13	-	-	-	-	-	>100[a]	>100[a]	>100[a]	>100[a]	100	-	-	-	-	-
O-F-14	-	>100[bc]	>100[bc]	>100[bc]	-	86[a] (75-98)	78[a] (64-84)	>100[a]	>100[a]	47	-	-	-	-	-
O-F-15	>100[a]	>100[a]	86[a] (72.9-92.0)	>100[a]	-	>100[a]	>100[a]	>100[a]	>100[a]	100	-	-	-	-	-
O-F-16	>100[a]	33.8[a] (21.2-60.7)	>100[a]	>100[a]	-	93.4[ab]	34.9[a] (21.5-47.8)	>100[a]	>100[a]	4.73	-	-	-	-	-
O-F-17	28.8[bc]	>100[bc]	26.2[bc]	-	-	20.4[a]	82.3[a]	10.6[a]	-	0.004 (n=3)	-	-	-	-	-
O-F-18	4.5[b] (1.2-5.1)	<25.0[bc]	8.1[b] (7.1-8.7)	36.3[b] (23.2-45.9)	-	6.9[b] (4.8-8.8)	>25.0[bc]	>11.0[bc]	35.4[b] (22.0-71.3)	0.29	-	-	-	-	-
O-F-19	>100[a]	>100[a]	>100[a]	>100[a]	-	>100[a]	>100[a]	>100[a]	>100[a]	100	-	-	-	-	-
O-F-20	4.7[bc] (4.0-5.7)	<1.6[bc]	46[bc] (38-58)	>100[bc]	-	-[b]	0.5[b] (0.5-0.7)	1.8[b] (1.2-2.1)	>100[a]	-	-	-	-	-	-
O-F-21	6.5[b] (5.0-7.6)	15.2[b] (13.8-16.0)	6[b] (5.0-7.0)	5.2[b] (4.9-5.5)	-	7.7[b] (5.5-9.9)	8.5[b] (7.2-9.6)	5.4[b] (3.6-6.7)	5.5[b] (3.5-6.8)	1.91	-	-	-	-	-
O-F-22	28[bc] (26-31)	<3.1[bc]	55[bc] (42-77)	35[bc] (29-43)	-	16[b] (14-17)	<3.1[b]	24[b] (21-26)	17[b] (13-20)	0.09	-	-	-	-	-
O-F-23	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[a]	>100[a]	>100[a]	>100[a]	100	-	-	-	-	-

TABLE B.3: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	Fathead Minnow										*Oncorhynchus mykiss*				
	Survival					Growth					Survival				
	IC25 (95% CI)					IC25 (95% CI)					IC25 (95% CI)				
	Quarters				PMTC	Quarters				PMTC	Quarters				PMTC
	1st	2nd	3rd	4th	(%)	1st	2nd	3rd	4th	(%)	1st	2nd	3rd	4th	(%)
O-F-24	-	-	-	-	-	5.8[p]	8.3[p]	>100[p]	>100[p]	$4x10^{-5}$ (n=3)	-	-	-	-	-
O-F-25	-	-	-	-	-	>100[p]	69.3[p] (65-73)	38.9[p] (33-43)	36.6[p] (29-43)	4.23	-	-	-	-	-
O-F-26	13.8[p] (8.9-19.5)	8.9[p] (6.7-11.8)	6.5[p] (3.4-8.3)	13.2[p] (9.4-17.2)	-	12.8[ns]	>50[ns]	25.4[p] (15.4-35.1)	17.5[p] (11.6-35.3)	0.99	-	-	-	-	-
Quebec															
Q-F-03	-	55[p] (50-60)	>33[p]	9.2[p] (8.0-10.2)	-	-	38.6[p] (35.3-42.2)	32.2[p]	12.1[p] (7.0-54.3)	0.13 (n=3)	-	-	-	-	-
Q-F-04	-	-	>100[ns]	>100[ns]	-	>100[p]	96.7[p] (93.2-99.5)	>100[p]	>100[p]	90.6	-	-	-	-	-
Q-F-05	-	46.4[ns] (33-100)	>100[ns]	33[ns] (11-100)	-	8.9[p] (5.8-16.3)	13.5[p] (5.8-18.7)	31.8[p] (10.7-44.6)	23.7[ns]	0.815	-	-	-	-	-
Q-F-06	3.5[ns] (3.0-4.3)	>100[ns]	37.3[ns] (31.1-45.1)	>100[ns]	-	-[p]	>100[p]	2.4[p] (1.1-3.0)	>100[p]	-	-	-	-	-	-
Q-F-07	-	-	26[ns&p] (11-100)	18[ns] (13.7-24.4)	-	2.4[p] (1.8-2.9)	3[p] (1.9-5.0)	2.1[p] (0.4-4.8)	5.9[p] (2.0-9.2)	0.261	-	-	-	-	-
Q-F-09	16.0[ns] (13.2-19.5)	15.2[ns] (12.5-18.8)	20.3[ns] (15.7-26.7)	>100[ns]	-	12.2[p] (8.1-21.9)	8[p] (6.8-10.6)	8.4[p] (6.9-10.7)	80.2[p]	1.35 (n=3)	-	-	-	-	-
Q-F-10	17.5[ns] (15.3-20.1)	18.0[ns] (16.7-19.4)	29.2[ns] (27.6-31.0)	>100[ns]	-	7.8[p] (12.4-28.8)	6.4[p] (4.4-13.6)	17.6[p] (13.6-20.2)	>100[p]	0.11 (n=3)	-	-	-	-	-
Q-F-12	4.5[ns] (2.8-6.6)	4.3[ns] (3.4-5.4)	>100[ns]	>100[ns]	-	-[p]	-[p]	>100[p]	>100[p]	-	-	-	-	-	-
Q-F-13	5.1[p] (3.4-5.8)	>100[p]	>100[p]	>100[p]	-	2.6[p] (2.1-3.3)	73.4[p] (57.2-86.1)	>100[p]	85.9[p]	23.47 (n=3)	-	-	-	-	-
Q-F-15	>100[p]	>100[p]	>100[p]	-	-	>100[p]	>100[p]	>100[p]	-	100 (n=3)	-	-	-	-	-

TABLE B.3: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	Fathead Minnow										*Oncorhynchus mykiss*				
	Survival					Growth					Survival				
	IC25 (95% CI)				PMTC (%)	IC25 (95% CI)				PMTC (%)	IC25 (95% CI)				PMTC (%)
	Quarters					Quarters					Quarters				
	1st	2nd	3rd	4th		1st	2nd	3rd	4th		1st	2nd	3rd	4th	
Q-F-17	2.9[de]	9.7[e] (5.1-16.9)	>100[e]	>100[e]	-	6.2[e] (3.1-7.6)	10.1[de]	66.9[e] (43.7-82.2)	45.8[e] (34.2-54.4)	$7.2x10^{-3}$ (n=3)	-	-	-	-	-
Q-F-18	-	>100[e]	>100[e]	>100[e]	-	-	>100[e]	>100[e]	82.5[e] (70.1-89.6)	36.98 (n=3)	-	-	-	-	-
Q-F-19	-	44.9[de] (25-100)	>100[be]	>100[be]	-	17.4[e] (13.7-22.7)	22.1[e] (15.1-29.8)	50.9[e] (16.4-77.5)	60.8[de] (44.7-77.3)	-	-	-	-	-	-
Q-F-20	-	-	<30.3[e]	-	-	3.3[e] (2.1-4.2)	0.9[e] (0.3-1.9)	>3.3[e]	-	$4x10^{-3}$ (n=3)	-	-	-	-	-
Q-F-21	3.2[be] (1.0-10.0)	1.9[be] (1.5-2.5)	>100[be]	>100[be]	-	1.4[e] (0.8-2.2)	0.9[e] (0.4-1.5)	>100[e]	98.4[e]	-	-	-	-	-	-
Q-F-22	-	22.6[be] (11.0-33.0)	2.1[be] (1.6-2.7)	>100[be]	-	12.7[e] (11.0-14.5)	11.9[e] (8.5-15.8)	-[e]	>100[e]	-	-	-	-	-	-
Q-F-23	>100[be]	87[be] (50-100)	>100[be]	>100[be]	-	82.1[e] (76.6-86.8)	-[e]	40.1[e]	>100[e]	-	-	-	-	-	-
Q-F-24	4.8[e] (3.7-5.5)	3[e] (2.6-3.7)	>100[e]	>100[e]	-	3.4[e] (3.0-3.4)	4.6[e] (3.2-5.8)	>100[e]	71.3[e] (55.8-86.2)	-	-	-	-	-	-
Q-F-25	87.5[e] (72.1-95.9)	>100[e]	>33[de]	>100[e]	-	33.5[e] (28.9-39.7)	>100[e]	23.9[e] (17.0-28.5)	>100[e]	0.991	-	-	-	-	-
Q-F-26	>100[e]	-	>100[e]	>100[e]	-	>100[e]	>100[e]	83.9[e]	>100[e]	59.74	-	-	-	-	-
Q-F-27	>100[be]	>100[be]	41.5[be]	>100[be]	-	>100[e]	>100[e]	-[e]	>100[e]	-	-	-	-	-	-
Q-F-28	15.9[be] (13.8-18.2)	<0.39[be]	6.2[be] (4.6-8.5)	4.58[be] (3.72-5.64)	-	9.5[e] (8.4-10.8)	<0.39[e]	2.6[e] (1.6-7.8)	1.99[e] (1.21-2.60)	0.0018	-	-	-	-	-
Q-F-31	-	-	18.5[bde] (11-33)	37.8[be] (11-100)	-	30.4[e] (26.0-34.8)	7.7[e] (6.3-9.4)	-	13.3[e] (<0-23.2)	0.044 (n=3)	-	-	-	-	-
Q-F-32	-	-	7.2[be] (3.7-11.0)	>100[be]	-	6.6[e] (5.7-7.3)	6.1[e] (5.4-6.6)	5.4[e] (3.9-6.5)	>100[e]	2.6 (n=3)	-	-	-	-	-

TABLE B.3: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	Fathead Minnow										*Oncorhynchus mykiss*				
	Survival					Growth					Survival				
	IC25 (95% CI)					IC25 (95% CI)					IC25 (95% CI)				
	Quarters				PMTC	Quarters				PMTC	Quarters				PMTC
	1st	2nd	3rd	4th	(%)	1st	2nd	3rd	4th	(%)	1st	2nd	3rd	4th	(%)
Q-F-34	-	3.7[bp] (1.2-11.0)	8.9[bp] (6.7-11.8)	>100[bs]	-	6.6[p] (5.6-7.3)	-	7.7[p] (5.3-12.8)	>100[s]	-	-	-	-	-	-
Q-F-35	-	-	>100[bp]	>100[bs]	-	40.9[p] (30.6-50)	16.6[p] (13.6-21.5)	61.6[p] (41.5-75.5)	>100[s]	0.126 (n=3)	-	-	-	-	-
Q-F-36	6.7[bp] (5.6-8.1)	3.7[bp] (3.0-4.7)	7.1[bp] (5.7-8.7)	4.4[bp] (3.6-5.3)	-	3.9[p] (3.0-5.2)	-[s]	[s]	2.7[p] (2.2-3.6)	-	-	-	-	-	-
Q-F-37	7.3[p] (6.9-7.8)	18.3[p] (17.0-19.6)	30.3[p] (25.9-31.8)	>25.0[bp]	-	4.9[p] (4.3-5.3)	11.6[p] (10.0-12.8)	8.5[p] (7.1-10.3)	8.4[p] (6.6-9.6)	1.168	-	-	-	-	-
Q-F-39	15.8[p] (14.4-17.0)	4.9[p] (1.2-6.1)	11.3[s] (9.8-45.9)	27.7[s] (16.0-36.5)	-	9.2[p] (7.6-10.7)	2.3[p] (0.4-4.3)	12.3[s] (9.1-27.6)	24.6[s] (14.3-35.1)	-	-	-	-	-	-
Q-F-42	55.4[bp]	27.7[bp]	>100[bp]	72.2[bp]	-	3.1[p]	>25[p]	>100[p]	-[s]	8.56X10^{-6} (n=3)	-	-	-	-	-
Q-F-44	5.5[p] (5.2-5.7)	2.6[p] (2.4-2.8)	>3.7[p]	5.9[p] (3.7-11.0)	-	3.7[p] (2.7-4.1)	1.8[p] (1.4-2.1)	2.3[p] (1.4-2.8)	1.2[p] (0.8-2.1)	0.1652	-	-	-	-	-
Q-F-45	24.5[p] (9.7-34.6)	2.5[p] (1.8-3.8)	5[p] (4.0-5.4)	23.5[s] (18.1-36.9)	-	12.8[p] (8.9-16.9)	3.4[p] (3.0-5.1)	3.1[bp]	22[s] (16.0-28.1)	6.6x10^{-3} (n=3)	-	-	-	-	-
Q-F-46	32.1[bp] (27.2-37.6)	6.8[bp] (5-10)	1.1[bp] (0.67-1.75)	5.1[bp] (3.7-6.7)	-	6.6[p] (4.7-8.9)	3.6[p] (1.7-6.0)	-[s]	-[s]	-	-	-	-	-	-
Q-F-47	<33.0[bp]	45.2[p] (36.7-52.4)	2.9[p] (2.0-15.9)	0.4[p] (0.2-1.1)	-	8.1[p] (1.7-19.4)	20.2[p] (11.9-24.0)	2.4[p] (1.8-3.4)	>11.0[bp]	0.068	-	-	-	-	-
Q-F-49	>100[bp]	54.4[bp] (33-100)	2.1[bs] (1.2-3.7)	54.4[bs] (48.1-61.6)	-	57.1[p] (51.1-62.1)	>33[p]	>1.2[p]	-[s]	-	-	-	-	-	-
Q-F-50A	-	55[p] (50-60)	>33.0[p]	9.2[s] (8.0-10.2)	-	-	38.8[p] (35.3-42.2)	32.2[p]	12.1[s] (7.0-54.3)	-	-	-	-	-	-
Q-F-50B	-	-	7.4[bsp] (3.7-100)	2.8[bs] (1.2-3.7)	-	24.9[p] (14.8-31.5)	22.8[p] (10.5-29.5)	-[s]	1.0[bs]	1.79X10^{-6} (n=3)	-	-	-	-	-

TABLE B.3: RESULTS OF SUBLETHAL FRESHWATER TESTS FOR CYCLE 1 EEM

Mill Name (Outfall)	Fathead Minnow										*Oncorhynchus mykiss*				
	Survival					Growth					Survival				
	IC25 (95% CI)				PMTC (%)	IC25 (95% CI)				PMTC (%)	IC25 (95% CI)				PMTC (%)
	Quarters					Quarters					Quarters				
	1st	2nd	3rd	4th		1st	2nd	3rd	4th		1st	2nd	3rd	4th	
Q-F-52	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	>100[a]	>100[a]	85[a]	>100[a]	62.07	-	-	-	-	-
Q-F-53	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	23.3[a] (19.0-53.0)	48.3[a] (40.1-84.5)	83.9[a] (71.7-92.5)	>100[a]	1.6396	-	-	-	-	-
Q-F-55	2.6[bc] (2.3-2.9)	2.0[bc] (1.7-2.2)	>100[bc]	>100[bc]	-	1.1[a] (1.0-1.2)	1[a] (0.8-1.1)	>100[a]	57.4[a] (39-71)	-	-	-	-	-	-
Q-F-58	54.8[a] (28.4-74.2)	56[a] (50-62.1)	68.2[a] (65.3-71.6)	2.9[a] (2.1-4.8)	-	53.2[a] (36.7-68.0)	11.7[a] (10.5-13.9)	67.9[a] (61.1-74.6)	3.7[a] (2.2-6.1)	0.013	-	-	-	-	-
Q-F-63	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	-	-	-	-	-	-	-	-	-	-
Q-F-64	>100[bc]	>100[bc]	>100[bc]	>100[bc]	-	26.7[a] (4.8-54.1)	>100[a]	18[a] (11.8-20.6)	3.6[a]	0.013	-	-	-	-	-
Atlantic															
A-F-01	-	-	-	-	-	-	-	-	-	-	>100[b]	>100[b]	>100[b]	>100[a]	100 (n=3)
A-F-08	31.7[a] (28.0-34.1)	>33.0[a]	>33.0[a]	>33.0[a]	-	26.1[a] (20.5-29.7)	18[a] (12.1-21.2)	21.9[a] (18.6-25.4)	20.7[a] (17.8-23.9)	9.41	-	-	-	-	-
A-F-12	-	-	-	-	-	32.6[a]	26.1[a]	21.5[a]	24.6[a]	10.2	-	-	-	-	-
A-F-15	-	-	-	-	-	71.4[a] (53-86)	>100[a]	>100[a]	>100[a]	37.2	-	-	-	-	-

[a] Complete mortality at higher concentrations
[b] LC50
[c] Bimodal response
[d] Estimated value
[e] Problem with statistical endpoint (i.e. IC25>LC50)
[p] Primary treatment
[s] Secondary treatment

Appendix C

- ***Industry/Government EEM Workshop Outputs***
- ***Specific Environment Canada Regional Problems and Recommendations***
- ***Feedback from Consultants at St. Andrew's EEM Symposium (October 1995)***

Industry/Government EEM Workshop Outputs

1. **Industry - Government EEM Workshop, May 27-30, 1996, Sidney, BC**

Key Points

- **Improving test sensitivity in sublethal fish toxicity test:** Sublethal fish toxicity tests (eg: inland silverside methodology) needs assessment due to lack of sensitivity at most mills in BC. Note that it showed toxicity to primary treated effluent but results changed to non-toxic following secondary treatment (eg: Port Alice). Substitutions for other sublethal fish test should be considered or different life phases eg: salmonid e-test is insensitive, and egg availability was a problem in Cycle One. E-A-F test protocols need development for use.

- **Reducing test variability of plant and invertebrate tests:** Plant and invertebrate tests were more sensitive but variable eg: *Champia* test needs evaluation due to variability. Problems with *Lemma* test, changing protocols midstream, lack of sensitivity. Fathead minnow used in Fibreco seems to be okay.

- **Changes in effluent character versus test variability:** Toxicity tests should be done quarterly because the effluents can change throughout the year depending on stock source. More frequent toxicity testing may be required to assess effluent variability using only those tests which work (sensitive, low variability). In addition, mills should be required to chemically characterize effluents used in each toxicity test period to help understand the relationship between toxicity test variability and effluent character variability. Tests used therefore need to be available year round. The Cycle One program had included quarterly sampling however supplier problem for the rainbow trout early life stage resulted in a shorter time frame to complete 4 sets of tests in Cycle One. Also, *Champia* test only available from 2 suppliers in Canada (one in BC and one in Ontario). Will toxicity testing be annual (eg: 4 times a year)?

- **Decision criteria and further guidance to interpret toxicity test results:** Need more guidance on what the results mean e.g. decision criteria for when action is required. PMTC interpretation does not provide useful information using present data, given the very low values obtained (<0 x %). PMTC is very sensitive to number of tests conducted. Alternative approach which provides meaningful data is to use IC25 data. IC25 information can be compared between tests and with receiving environment effluent dilutions.

Next Steps

- National toxicity test database of results needs evaluation to refine or replace tests which are variable, insensitive or not available year round. The meaning of sensitivity or toxicity needs be addressed within a decision framework to guide interpretation. Working group to be formed.

2. **Industry - Government EEM Workshop, September 10-11, 1996, Downsview, ON**

Specific Recommendations

- Toxicity testing should remain as part of the Cycle Two core list.

- For the fathead minnow test, the NOEC, LOEC and the threshold effect concentration endpoints should be removed and only the LC50, IC50 and IC25 endpoints be retained. This would simplify the test requirement with no apparent loss of information.

- The fathead minnow growth and survival endpoints seem to be equally sensitive. Further evaluation is required to see if one endpoint can be eliminated.

- The duckweed test has been demonstrated to be less sensitive than the *Selenastrum* test. It is recommended that the *Selenastrum* 72 h test replace the duckweed test.

3. **Industry - Government EEM Meeting, December 10, 1996, Fredericton, NB**

Specific Recommendations

- Sublethal toxicity tests should be retained as a core element (EWG should assess which tests/organisms and assess frequency).

- Consider using mill specific IC25 test in a tiered approach where the frequency of testing is based on the magnitude of effects.

- Eliminate Duckweed and use *Selenastrum* for fresh water studies.

- Improve/replace *Champia* test for marine receiving water.

- Determine the quality of relationship between measures using non-indigenous species and actual effect on indigenous species.

- PMTC should be used as an option only (not core indicator).

4. **Alberta Forest Products Association, "Summary of Cycle 1 EEM Studies", January, 1997**

Issues

- **Fathead minnow larval growth test:** The effluents had no effect on fathead minnow (*Pimephales promelas*) larval growth. These non-toxic results were consistently observed both among mills over time.

- **Invertebrate reproduction test:** The PMTCs for invertebrate (*Ceriodaphnia dubia*) reproduction were 20% or greater for a number of mills (3 mills), and the test data were remarkably consistent over the entire monitoring period for these same mills. At these mills, the variation in the IC25s and the IC50s is consistent with that observed for most biological tests. At four mills, the NOECs for reproduction were for the most part 25 or 50%. In other words, dilution by half renders the effluent non-toxic. As this species is incredibly sensitive to many different stressors (chemical, physical, and biological) these results would tend to indicate that negative environmental effects are not expected.

- **Algal growth test:** The effect on algal (*Selenastrum capricornutum*) growth is real and likely chemical in nature since a one-in-twenty-fold dilution was at times required to render the effluents non-toxic (as evidenced by the low PMTCs).

Recommendations

- **Fathead minnow larval growth test:** Due to the consistent lack of effects, this test should be dropped from future cycles.

- **Invertebrate reproduction test:** For several mills, additional monitoring will not provide any useful information because of the consistency in the test results and lack of any indication of an effect in the field. The effect on invertebrate reproduction at these mills is too marginal for a TIE (toxicity identification evaluation). Additional monitoring will only be useful at mills which have had inconsistencies in their results thus far.

- **Algal growth test:** Because of some inconsistencies in the results, the algal test should be conducted for another cycle.

General Recommendations

- The concept and usefulness of toxicological testing should be re-thought. These tests do not account for the amount or volume of effluent flow. As mills reduce their overall effluent discharges, the remaining streams may become more concentrated. Without considering dilution effects and overall loadings, these tests will give a false indication of the environmental effects.

5. **Industry - Government Regional Coordinators Meeting, February 4, 1997, Toronto, ON**

Specific Issues

- Toxicity should be retained as a core element.

- Rationale for doing tests:

  - To compare effluent quality between mill types and to measure changes in effluent quality as a result of effluent treatment and process changes;
  - To determine the relative contributions of multiple discharges to effects observed in field studies (basin approach);
  - To assess the potential of an effluent to have a negative impact on the receiving environment.

- EWG must develop a decision tree (see general principles).

- EWG must identify ecological significance of the results.

- EWG must recommend on the frequency of testing (eg: decrease frequency if non-toxic or increase if toxic) and reporting.

- EWG should develop a check-list for QA/QC (validation test) for mill use. (Some work has already been done by the EWG).

- EWG is asked to investigate the possibility/practically of testing the receiving waters (control and dilution).

- EWG should identify joint industry/government pilot studies for new methodologies.

6. **AIFQ (Association des Industries Forestières du Québec) - Environment Canada Eem Symposium for Quebec Region, March 20, 1997, Montreal, Quebec**

Minutes from this Symposium are not included in that report since they were still under review.

Specific Environment Canada Regional Problems and Recommendations

1. **Pacific and Yukon Region (memorandum: J. Boyd to R. Scroggins; November 25, 1996)**

Issues

- **No results - Paradoxical dose response:** Some mills report no results for some quarters due to a paradoxical dose response. Did this happen in other regions? We will be requesting this missing results from the mills.

- **Miscellaneous problems with specific tests:**

  - Refresh not done or not proper date;

  - Short time between testing (2 weeks, 1 week, 5 minutes);

  - Should have lower dilution.

- **Tests not done for each quarter or tests not done on the same effluent:** These generally occurred either because of individual tests failures where tests had to be rerun or rainbow trout embryos were not available at certain times. Mills waiting for rainbow trout supply to run tests with the same effluent could not test in each quarter. Those that proceeded with the plant and invertebrates tests then ran the rainbow test at a different time therefore different effluent.

2. **Pacific and Yukon Region: Summary of Major Sublethal Toxicity Testing Issues Identified During EEM Cycle One Data Review (J. Bruno, February 12, 1997).** Abbreviated from a 44 page report by J. Bruno.

- Starting tests with effluent samples which were greater than three days old.

- Failure to meet (or report) all defined tests validity criteria.

- Not following the "must" requirements of the test method (eg: use of minimum number of test organisms, running the test longer than allowed, etc.).

- Failure to re-sample and repeat the test when validity criteria have not been met in original effluent test.

- Failure to conduct a reference toxicant test in a similar manner to the effluent test (ie: 48hr reference toxicant test when the effluent test is 7 days in length).

- Use of supersaturated water for test controls and effluent dilutions.

- Pre-aerating or aerating test solutions contrary to method recommendations.

- Failure to report essential test condition information (eg: not reporting number of eggs and volume of sperm per test vessel in the sea urchin fertilization test.

3. **Ontario Region**

Data Quality

- Storage temperature for some effluent samples was 15 degrees, well outside the range of 1-7 degrees C recommended by the standard methods.

- A number of *Ceriodaphnia dubia*, and fathead minnow tests were not initiated within 72 hours of sampling.

- Some fathead minnow growth tests exceeded the Minimum Significant Difference in growth; some *Selenastrum capricornutum* growth tests had less than acceptable growth in the control.

- Some *C. dubia* tests resulted in significant or complete mortalities in the control populations, or the mortalities recorded in the control populations exceed those in the adjacent lower effluent concentrations.

- Some of the control samples for *L. minor* exposures failed to double the number of fronds in the four days of exposure as required.

- The data for some of the tests at some mills demonstrated a great deal of variability from one sampling period to another with no distinguishable pattern.

Relationship between laboratory and field results

- Some mills concluded that there was a lack of data (3 tests x 4 times) to draw any conclusions about sublethal toxicity in the laboratory and possible effects on plant, invertebrate or fish species in the receiving environment.

- Other mills concluded that their effluent may have the potential to cause sublethal toxicity effects on sensitive plant and/or invertebrate and/or fish species throughout their receiving environment.

Recommendations

- Wherever sublethal toxicity was evident in a mill's tests and/or there was high variability in the results obtained and/or there were a number of invalid tests and/or secondary treatment was installed following the four rounds of tests, the TAP has recommended that toxicity testing should be repeated in Cycle Two.

- For the fathead minnow test, industry/government in Ontario have recommended the NOEC, LOEC and the threshold effect concentration endpoints should be removed and only the LC50, IC50 and IC25 endpoints be retained. This would simplify the test requirement with no apparent loss of information.

- Fathead minnow growth and survival endpoints seem to be equally sensitive, therefore industry/government in Ontario have recommended further evaluation is require to see if one endpoint can be eliminated.

- The duckweed test was demonstrated in a number of cycle 1 studies to be less sensitive than the *Selenastrum* test. Ontario industry/government have recommended that the *Selenastrum* 72 h test replace the duckweed test.

4. **EEM Working Group Workshop, December 13, 1996, Aylmer, ON**

Pacific and Yukon Region

Constraints

- Lack of data (3 tests x 4 times).
- Lack of criteria (cycle 1 to provide).
- Lack of sensitivity.
- High variability in test.

Alternatives

- Variability assessment (effluent characterization).
- Modifications / other toxicity tests.
- TIEs.
- Replacement of acute toxicity tests.

Quebec Region

Quality Control of Toxicity Tests

- Some IC25 or 50 <NOEC after extrapolations and some ICs>LCs.

Recommendation: Increase the number of dilutions for each sublethal toxicity test.

- Too long delays (up to 90 days!) between tests of effluents and tests of reference toxicants.

Recommendation: Limit the duration to 8 days.

Results of Toxicity Tests

- The results of duckweed tests were generally not positive.

Recommendation: Softwater plant tests only with algae.

- The results of sublethal fish toxicity tests were generally less sensitive than the results of sublethal plant of invertebrate toxicity tests.

Recommendation: Priority to plant and invertebrate tests.

- Tables with 5 or 10 endpoints for each toxicity test do not facilitate understanding.

Recommendation: Limitation to sublethal LOEC, NOEC, and TEC in tables and possibility to express in sublethal toxic units (TU).

- The prediction of sublethal threshold for receiving environment produced very low questionable values.

Recommendation: Possibility to do dilutions with water of receiving environment for toxicity tests.

Atlantic Region

- 4 freshwater and 13 marine mills.
- *Lemna* test was not sensitive.
- *Champia* tests sensitive but had poor reliability.
- Data reporting was deficient.
- Reference toxicant testing incomplete.

Conclusions

- Need to be repeated because of changes in effluent quality.
- *Champia* tests should be replaced.
- Drop *Lemna* test
- Good correlation with ICS (Invertebrate Community Structure) results.
- ELS (early life stage) fish test did not correlate with AFS (Adult Fish Survey).
- Frequency of testing.

Feedback from Consultants at St. Andrew's EEM Symposium (October 1995)

1. **B.A.R. Environmental, Inc. Presentation: "Toxicological Testing" (K. Holtze *et al.*)**

Recommendations

- **Issue of single vs multiple samples**
  - Leave options open with "should" as opposed to "must".
  - Start with simple design (single sample) and work to more complex design (multiple samples).

- **Issue of sample handling conditions during transport**
  - Minimize time between sample collection and delivery to testing laboratory.
  - Study effects of rapid cooling on effluent toxicity.
  - Determine need for refrigeration.
  - Ice paks may not be a "risk free" option.

- **Issue of age in fathead minnows used in sublethal tests**: Consider amending protocol to permit use of older fish (1 to 4 days old), but within a narrow age limit (eg: 24 hrs).

- **Issue of reference toxicant testing for the E, EA, and EAF tests:** Consider using a common age group and exposure period for all three test methods.

- **Issue of test endpoints:** Exclude inappropriate endpoints.

2. **Beak Consultants Limited Presentation: "Evaluation of Duckweed Method" (E. Jonczyk and G. Gilron)**

Recommendations and Conclusions

- **Standardization and further development of the test method is required** (Van Steertegem and Persoone 1993). Specific points to consider are:

  - Determination of the test precision is recommended.
  - A selection of reference toxicants for quality control requirements should be provided.
  - Determination of test organism sensitivity to various toxicants and complex effluents in comparison with other test species should be conducted.
  - Determination of the test's usefulness and application to regulatory purposes should be closely evaluated.

- **Standard development of the test under the "Biological Test Method" Publication Series** should be undertaken and a new, standardized test method adaptation at the national/international level in a regulatory context should also be considered.

- **Inter-laboratory evaluation ("round robin") testing of the standardized test method** should be conducted. This exercise would help to clarify the precision of the test, and will promote its use in the scientific community.

- **Inter-laboratory evaluation ("round robin") testing of the standardized test method**

3. **Beak Consultants Limited / EVS Environment Consultants Presentation: "EEM Issues to be considered for Cycle Two (M. Mudroch and G. Gilron)**

General Issues

- **Use of refresh samples for tests of ≥3 days duration**
  - PRO: Science, program goals (evaluation of effluent effects integrated over time).
  - CON: Scheduling, logistics, increased shipping and testing costs.

- **Pre-filtration of sample**
  - PRO: Necessary if sample has organisms which may compete with or attack test organisms, or which may be confused with the test organism.
  - CON: May alter toxicity (not likely for fish tests; possible for algal tests).

- **Aeration:** Must aerate if dissolved oxygen concentration drops below 40% saturation for a chronic test but low dissolved oxygen signals toxic effect of sample.

- **Replication:** Should be at least four replicates per concentration to allow for default to non-parametric statistical tests.

- **Reference toxicant testing:** Chronic for chronic tests and acute for acute tests (assesses test system) or acute for all tests (assesses test organisms).

Freshwater EEM Tests

Salmonid Early Life Stage (Environment Canada, 1992a)

- **Embryo (E) test endpoint definition needs to be standardized**
  - Fertilization and mortality endpoints not reliably determined.
  - "Embryo viability" can be determined.

- **Reference toxicants**
  - Variability in response.

- Difficulties with Zn, phenol (repeatability).
- Cu, SDS work but results are variable.

- **Timing form fertilization to exposure to sample**
  - Exposure time is very short - occurs in time frame (minutes) between fertilization and water-hardening, after this there is very little interaction between egg and solution.
  - Will vary with day, technician, number of samples to set up.
  - Critical to standardize within and among labs - consider washing in test solution just before seeding replicate.

- **Gametes not available all year round (nationally)**

- **E test design impractical for routine use on multiple samples**
  - EVS has developed alternative design using 500 mL test volume per replicate and no egg baskets.
  - 4 replicates/concentration needed for non-parametric analyses.

- **Relative sensitivity of E, EA (embryo-alevin) and EAF (embryo-alevin-fry) endpoints**
  - Inter-laboratory comparison needed (E is least expensive but may also be least sensitive).
  - Validation needed of several aspects of testing procedure.

***Ceriodaphnia dubia* Survival and Reproduction (Environment Canada, 1992b)**

- **Use of limited age window (8 hr) for animals used to initiate the test**
  - Increased cost of test to labs because increase in monitoring time of broodstock.
  - Has not been demonstrated that this criterion is important.

- **Test duration clarification:** 7 ± 1 days or 6-9 days? Specific duration determined by when 60% of females have had ≥3 broods or when 60% of females have had ≥3 broods AND at least 15 young per female?

***Selenastrum capricornutum* Growth (Microplate method; Environment Canada, 1992c)**

- **Absorbance method for determining growth not suitable for testing effluents:**
  - All effluents can be assumed to have colour - interferes with absorbance.
  - Procedure to remove colour prior to measuring absorbance results in a loss of some cells
  - therefore must also be done on test control.

- **Coulter counter or manual counting method recommended**

- **Volatile compounds:** May be present and may result in significant growth inhibition in test control plate relative to QA plate - this would invalidate test.

- **Some confusion has arisen as to whether the test duration can be extended** beyond 72 hr, however Environment Canada has indicated that the duration is strictly 72 hr.

- **Errata to be published by Environment Canada regarding growth in test control plate:** It must not be significantly lower than growth in QA control plate.

***Lemna minor* Growth (APHA, 1992)**

- Test method is still a proposed method; validation has yet to be conducted
- Test has not been demonstrated to be sensitive to pulp and paper mill effluents relative to other freshwater sublethal tests

Marine Tests

- **Salinity adjustment of samples:** Use brine (standardize method) of brine generation and adjustment, or use dry sea salts (inconsistency of quality from batch to batch and across suppliers). Sometimes trace elements and trace contaminants cause a toxic effect, especially if a high concentration of sea salts is needed for salinity adjustment.

- **Statistical analyses - which control to use for comparison with test concentrations**: Compare salinity control (brine or sea salts) to negative control (dilution water) and pool (pooled control) if not significantly different? Compare test responses to salinity control or to pooled control or to negative control?

Echinoderm Fertilization (Environment Canada, 1992)

- **Seasonal variability in test organism used for testing depending on the spawning season (typically) *Strongylocentrotus purpuratus* or *Dendraster excentricus)***

- **Differences in sensitivity among species being used?**

- **Are continuous spawners an option (e.g. *Lytechinus pictus*)?**

- **Ecological relevance of species used?**

***Champia parvula* Reproduction (USEPA, 1994)**

- **Cultures very sensitive to water quality**: This is a good quality for a toxicity testing organisms but can make culturing troublesome.

- **Fertilization sometimes a problem.**

- **Availability of healthy cultures from suppliers sometimes a problem.**

- **Test sensitivity:** Poor because variability within and among replicates is high and obscures differences among test concentrations (often cannot detect 40-50% difference from the control).

- **Appears to be more sensitive to some effluents than the other tests**; will be a good test if we can get a handle on the above.

- **Number of replicates:** USEPA method specifies 3 but need to use at least 4 to be able to default to non-parametric tests.

Paradoxical Concentration Response Curves

- Occasional paradoxical concentration response curves are produced from a test:
  - How often do these occur ?
  - Are the curves most often seen in one test or specific to particular effluents ?
  - Are these curves "real" (= repeatable) ?; to determine if a response is real, either include more replicates, or conduct duplicate concurrent tests; in addition, running more concentrations will define the curve better.
  - If the curves are real, what are the underlying mechanisms ?
  - Is it valid to report statistical endpoints for such curves ?

Appendix D

Comments Received After the Last EWG Meeting on Specific Objectives and Rationale for the Use of Sublethal Toxicity Tests

Comments Received After the Last EWG Meeting on Specific Objectives and Rationale for the Use of Sublethal Toxicity Tests

A suggested change by Pierre Martel to EWG consensus on the toxicity test rationale statement in Section 4.2.1 is as follows:

- "The EEM program is designed to assess the adequacy of the regulation by examining fish (fish survey), habitat (benthic community survey) and the use of fish resources (dioxins and tainting) in the receiving environment. In this program the primary role of laboratory toxicity tests is to provide information about the quality of pulp and paper mill effluents as a supplement to the information furnished by measurements of the regulatory parameters (ie: BOD, suspended solids and the acute lethal test). The response of aquatic organisms to effluents obtained under controlled conditions can be used to quantify and compare the toxicological characteristics of mill effluents over time".

A suggested change by Valerie Naish to EWG consensus on the third specific objective for use of sublethal toxicity tests in Section 4.2.1 is as follows:

- "To assess the potential impact of an effluent on the receiving environment (research is needed to establish links between laboratory and field data before this objective can be met).

Appendix E

List of Issues Discussed at November 1996 PAPRICAN Videoconference

List of Issues Discussed at November 1996 PAPRICAN Videoconference

Issues Related to Effluent Sample Collection and Transport

- Time between sample collection and sample initiation: change "**must**" criterion of **72 h** "**must**" criterion of **3 days**;

- Cooling sample in transit: possible revision to "all samples **should** be kept cool throughout their period of transport and storage. As necessary, gel packs or other means of refrigeration..." to a **must** criterion and give examples of ice packs (with non-toxic contents) which could be used (eg: Blue Ice Rubbermaid, Polar Pak Mid-Lands or Johnny Plastic-Ice);

- **One** vs **three** discrete effluent samples for a static renewal tests (sheepshead minnow, inland silverside, fathead minnow, ELS salmonid and *Ceriodaphnia*).

Generic Test Method Issues

- **Pre-filtration** of sample: if this necessary?, for example, if a sample has organisms which may compete with or attack test organisms, or which may confused with the test organism, but it may alter toxicity (eg: *Ceriodaphnia* test);

- **Aeration** of sample: should there be aeration if dissolved oxygen concentration drops below 40% saturation for a sublethal test? Low dissolved oxygen signals toxic effect of sample;

- Replication: make **4 replicates** per concentration a "**must**" criterion. Default to non-parametric statistical tests anyway;

- **Reference toxicant** testing: should be a standard approach to reference toxicant testing for sublethal toxicity tests; Reference toxicity test design must match the effluent sample test design;

- **Lighting**: need to set minimum level of light intensity. Possibly express as a range (eg: 300 to 500 foot/candles);

- Marine tests, question of **salinity adjustment**: use brine or dry sea salts? Which control to use for comparison with test concentrations? Compare salinity control (=brine or sea salts) to negative control (=dilution water) and pool (=pooled control) if not significantly

different? Compare test responses to salinity control or to pooled control or to negative control?

- Minimum **level of reporting**: must be established (eg: report culture health information);
- Too many required **endpoints to report**: NOEC, LOEC, TEC, IC_{50}, IC_{25} and LC_{50} where appropriate;
- **Statistics**: Environment Canada's sublethal test protocols recommend Dunnett's procedure and William's test as parametric methods for the derivation of an LOEC. It is unclear, however, which is the preferred method. It would be helpful if information regarding the underlying assumptions of the various tests were presented as a rough guide to choosing the most appropriate method for a particular set of data;
- **MSD**: in making regulatory decisions, are MSD values taken into consideration when comparing LOECs? If an MSD is too high, are there statistically sound methods of treating the data to remove some of the more unmanageable variability? It would be extremely helpful if general guidelines regarding data analysis were provided in the main statistics manual (a simple list of "don'ts" might be appropriate).

Specific Test Method Issues

Inland Silverside Survival/Growth

- No specific methodology concerns;

Champia parvula Reproduction

- Test **sensitivity** is poor since variability within and among replicates is high and obscures differences among test concentrations (often cannot detect 40-50% difference from the control);
- Number of **replicates**: U.S.EPA method specifies **3** but need to use at least **4** to be able to default to non-parametric tests;
- Unclear **control validity** criterion for reproductive test: change "the test is acceptable if ... control plants average 10 or more cystocarps per plant" vs "control plants should average 10 or more cystocarps per plant in each replicate";
- **DO** measurements: dissolved oxygen measurements should not be required (add an unnecessary cost to the procedure). The plants produce oxygen in the light;
- Need for greater number of **male branches** at test initiation?

- Need for test vessel **shaker** system?

Echinoderm Fertilization Inhibition

- **Seasonal variability** in test organism used for testing depending on the spawning season (typically *Strongylocentrotus purpuratus* or *Dendraster excentricus*). Differences in **sensitivity** among species being used? Are continuous spawners an option (e.g. *Lytechinus pictus*)? **Ecological relevance** of species used?

Fathead Minnow Growth/Survival

- The growth and survival endpoints seem to be equally sensitive. Further evaluation is required to see if one **endpoint** can be eliminated;

- Possibility of greater flexibility needed with regards to **larvae age** at test initiation?

- Need guidance on how to treat growth data from replicates with **low survival** when calculating the ICp value;

- ICPIN program: how to treat replicates with **100% mortality**? Should that replicate be left blank (resulting in an uneven number of replicates in the test) or should a zero be entered (although a zero value does not represent the initial weight of the fish in that replicate but represents survival only)? What value should be entered when a concentration suffers complete mortality?

- Need to increase **minimum control growth validity** criterion;

- **Randomization**: since survival and growth are not greatly influenced by slight variations in environmental conditions, the use of randomized positioning is excessive. To minimize variation in feeding, use a repeater pipette to deliver artemia, adding the slurry by replicate, rather than in order of treatment;

- **Treatments with statistically significant mortality**: the statistical analysis for growth should exclude these treatments. This way, the endpoint for growth is separated from the mortality endpoint. This is a more accurate expression for a sublethal effect. That is, a toxicant which is acutely toxic, but has no effect on growth in a 100% treatment would be expressed as NOEC = 50% for mortality, NOEC > 50% for growth.

Selenastrum capricornutum Growth Inhibition

- **Absorbance** method for determining growth not suitable for testing effluents: all effluents can be assumed to have color - interferes with absorbance. The procedure to remove colour prior to measuring absorbance results in a loss of some cells - therefore must also

be done on test control. Coulter counter or manual counting methods should be recommended;

- **Culturing**: recommend that the stock culture remain separate from the culture that cells are harvested from for testing;

- **Quality of test organisms**: the culture appearance is a more rapid indicator of health than performing labour intensive monthly growth curves using several inoculum concentrations. For example, the colour of the culture (yellowing) and the appearance of precipitation of algae in the culture jar can indicate declining health. Additionally, by routinely taking absorbance measurements of the culture at 7 days, we can monitor the final growth yield over time. Substantial decreases in the absorbance reading of the culture is a simple indicator of poor culture health;

- **Washing of glassware**: recommend that even disposable glassware should be prewashed before use, especially if used to make dilutions;

- Preparation of the **enrichment medium**: suggest to reduce the EDTA and iron to be stoichiometrically in balance (20 mg/L $FeCl_3 \cdot 6H_2O$). Suggest to decrease the test to 48 h in order to avoid the CO_2 depletion and the pH drift of the medium;

- Preparation of **algal inoculum**: recommend that algae be washed and diluted with solutions with nutrient concentrations equivalent to the test solution (as opposed to $NaHCO_3$ solution);

- Use of **algal cells washed**: since the harvested cells are diluted to obtain the required cell density for inoculating the test solutions, the actual concentrations of dead cells and waste material in the nutrient solution is small. No statistically significant differences were found between two types of inoculum (washed and unwashed cells) conducted with three reference toxicants. Washing the algal inoculum is unnecessary;

- Preparation of the test solutions: recommend that a **pH** be chosen to conduct testing or, at the very least, that the test sample be adjusted to a pH equivalent to the control/dilution water;

- Procedure for **incubation**: recommend that if the microplates are going to be sealed in plastic bags, extra bicarbonate may be added to the medium and additional CO_2 may be added to the bag before sealing to minimize shift in pH;

- **Quality control** microplate: in order to evaluate a difference in cell yield, the control cell density from the three replicate test plates should be compared to the quality control plate by a t-test. This cannot be done with a single quality control plate, since replication of each plate is required to perform a t-test. A minimum of three replicate quality control

plates would be required to accurately assess differences in cell yield. In order to avoid problems with statistical analysis of a quality control plate and the extra space requirement to include the extra plates, use a different microplate configuration. The controls are places such that one row is beside the 100% treatment, one beside the 50% treatment, and one beside the 6% solution. If the controls are influenced by **volatile compounds**, the variability amongst the three rows would be high, and should exceed the required coefficient of variation of less than 20%, resulting in an invalid test;

- Test observations and measurements: recommend that **fluorometry** be added as an indirect method of estimating cell concentration;

- Test endpoints and calculations: recommend that **growth rate** be used to calculate inhibition, rather than **cell yield**;

- Validity criterion on differences between controls in test vs quality control microplates: significant difference in microplate controls seen in a pulp mill sample which seems to suggest that the stimulatory effect observed in the test plate controls might be due to an unknown volatile contaminant. Validity criterion needs to reflect possibility of **stimulation**;

- Growth in test control plate: revise wording in **validity criterion**, must not be significantly lower than growth in QA control plate.

- **Test acceptability**: pH is an inappropriate criterion for test acceptability since the small volume available in the microplate test prevents measuring pH to a high degree of accuracy (litmus paper is usually used). The test itself is designed to result in minimal changes due to CO_2 depletion.

Ceriodaphnia dubia Survival/Reproduction

- **Significant mortality**: the U.S.EPA recommends using Fisher's Exact test as a means of identifying significant mortality. A print out of this test is 3 pages in length and invariably confirms what the analyst has already determined by eye. There is also available a chart of mortality comparison data which summarizes the significance of differences between proportions. In cases where there is an equal number of replicates per test, this table would be a quicker, more convenient and environmentally friendly way to confirm significance rather than Fisher's test;

- **ICp derivation**: if we are to combine the mortality and reproduction data in calculating the ICp value, as defined by both the U.S.EPA and Canadian methods, then why would we also calculate an ICp value based on mortality alone? Page 27 in the test method specifies that when analysing mortality data by the ICPIN method, a concentration suffering complete mortality is excluded from the analysis. This is confusing because it instructs us

to use mortality data only and to omit a concentration suffering complete mortality, even though the ICPIN method specifically states that all concentration data are to be included;

- **Endpoints and calculations**: the two endpoints (**reproduction** vs **mortality**) should be distinguished for each other, so that dead adults are removed from the total number of adults producing young. In this manner, a sample would be considered only acutely toxic, if a true reproductive effect is not observed;

- Use of limited **age** window (8 h) for animals used to initiate the test: increased cost of test to labs because increase in monitoring time of broodstock. Any flexibility possible?

- **Test duration**: endpoint when 60% of females have had ³ 3 broods or when 60% of females have had ³ 3 broods **and** at least 15 young per female?

Lemna minor Growth Inhibition

- APHA test is **still a proposed method**: validation has not yet been conducted. Determination of the test precision is recommended. A selection of reference toxicants for quality control requirements should be provided. Determination of test organism sensitivity to various toxicants and complex effluents in comparison with other test species should be conducted. Determination of the test's usefulness and application to regulatory purposes should be closely evaluated;

- **Discrepancies** in testing techniques: either static, static-renewal or continuous-flow regimes may be used, depending on test solution stability;

- **Chamber size**: plants require certain volumes and water surface area for adequate growth. Provision of larger test vessels (100 mL instead of 50 mL) yields improved growth and therefore validity criteria are more likely to be me;.

- **Depth of test solution**: simpler to use plants with roots and test organisms must be provided a minimum of 5 cm depth of test solution;

- **Light intensity**: tests performed under lower light intensity (2150 lux) barely passed validity criteria, while plants tested with high light intensity (4300 lux) had a fourfold increase in growth;

- **Endpoint determination**: as the method describes that every protruding bud must be counted, the light table providing lighting from underneath the observation vessels should be used as the most reliable and repeatable methos for frond counts.

Trout embryo (E-test)

- Changes to salmonid method are well underway. Second edition to be published in early 1997.